

[Non haemolytic Staphylococcus]
white non haemolytic Colonies On Sheep blood agar



[B haemolytic Staphylococcus]

Yellow Colonies Surrounded by B haemolytic Zones on Sheep blood agar



[mannitol Salt agar for Staphylococci]

Selective → 7.5% Salt

differential → mannitol Sugar

indicator → phenol red

S. Aureus → ferment mannitol and Cause Phenol red to turn Yellow





A petri dish containing a bacterial culture on a red agar medium. A large, irregular, dark brown to black area of precipitation is visible on the left side of the dish, representing iron sulphide. The rest of the agar is a uniform red color.

[*Streptococcus equi*]

alpha haemolysis

H₂S production + iron → iron Sulphide [Black]

S, Viridans [a haemolytic Streptococci]

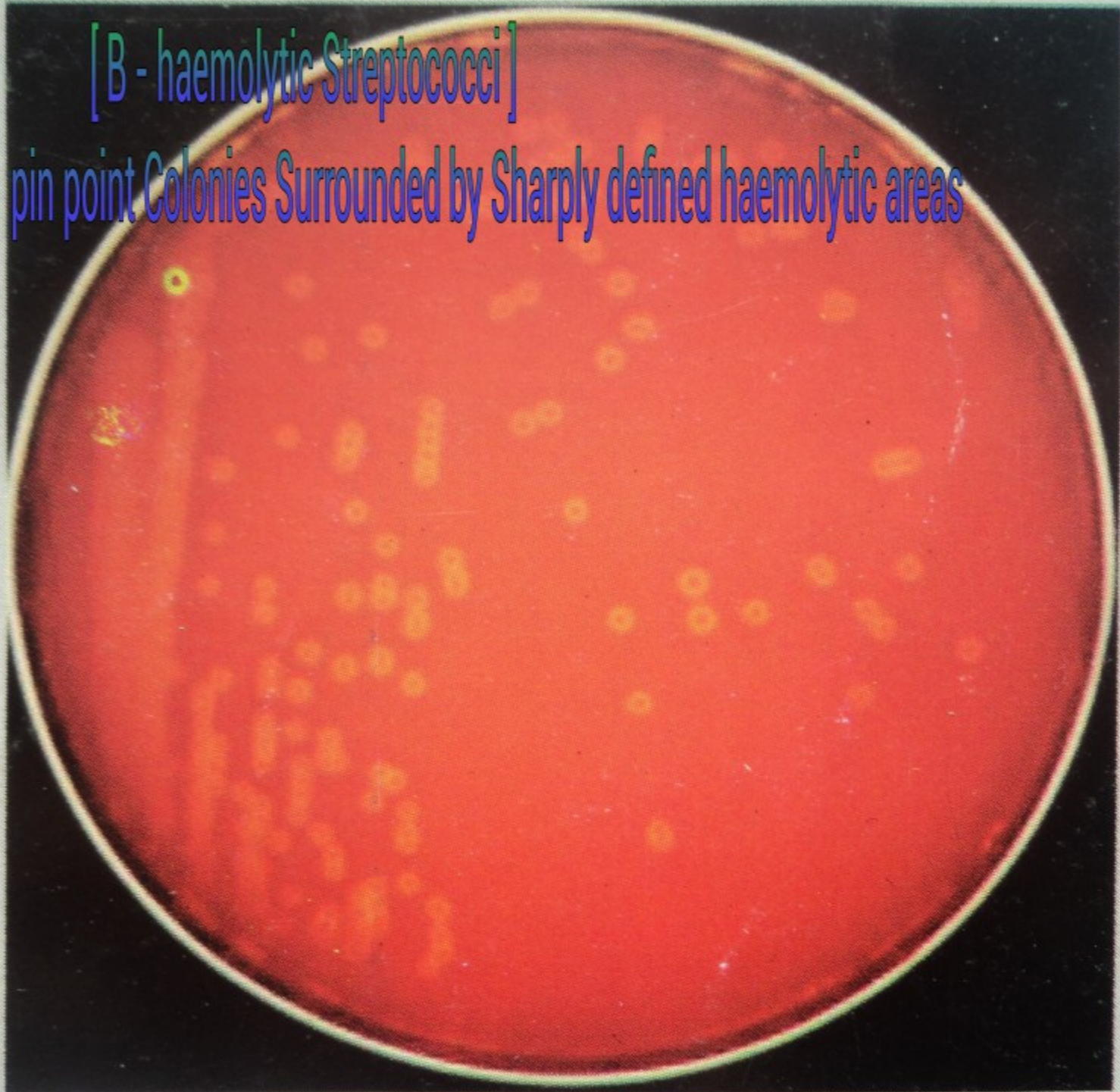
Colonies Surrounded by narrow Green Zones

H₂O₂ + haemoglobin → met haemoglobin [green]



[B - haemolytic Streptococci]

pin point Colonies Surrounded by Sharply defined haemolytic areas

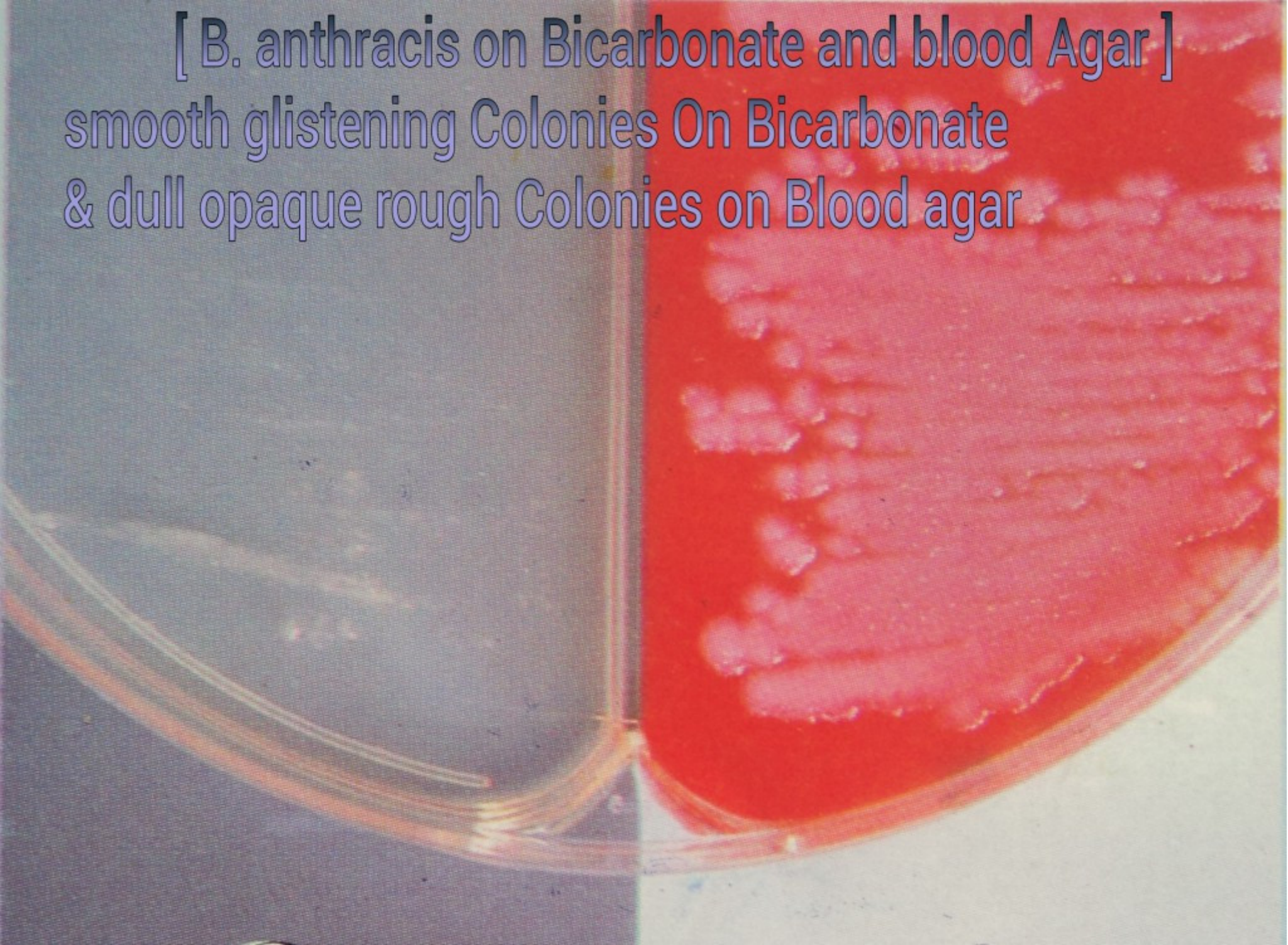


[Enterococcus agar for *E. fecalis*]

Contain Sodium azide → inhibit gram -ve bacteria
tetrazolium chloride reduced to formazon [red]

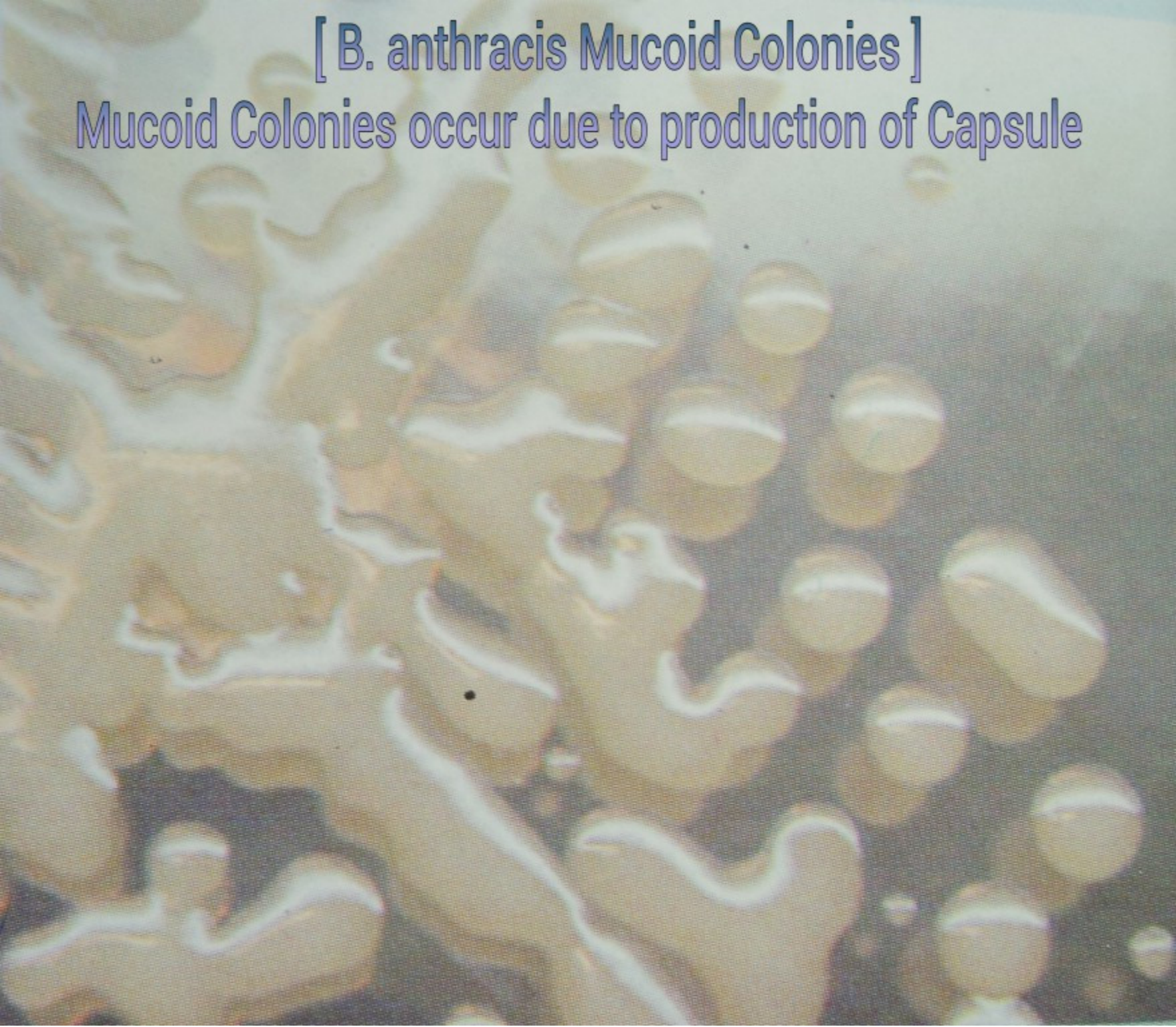


[*B. anthracis* on Bicarbonate and blood Agar]
smooth glistening Colonies On Bicarbonate
& dull opaque rough Colonies on Blood agar



[*B. anthracis* Muroid Colonies]

Muroid Colonies occur due to production of Capsule



[*B. Cereus*]

Colonies with Wide Zone of β haemolysis on Blood agar



[*B. Subtilis*]
narrow Zone of B haemolysis



B. Cereus [BCSA → Bacillus Cereus Selective agar]

BCSA media Contain :

Polymyxin B → inhibit gram - ve bacteria

PH indicator → Bromothymol Blue

mannitol → no fermentation

egg Yolk → opacity around Colonies



B. Subtilis [BCSA → Bacillus Cereus Selective agar]

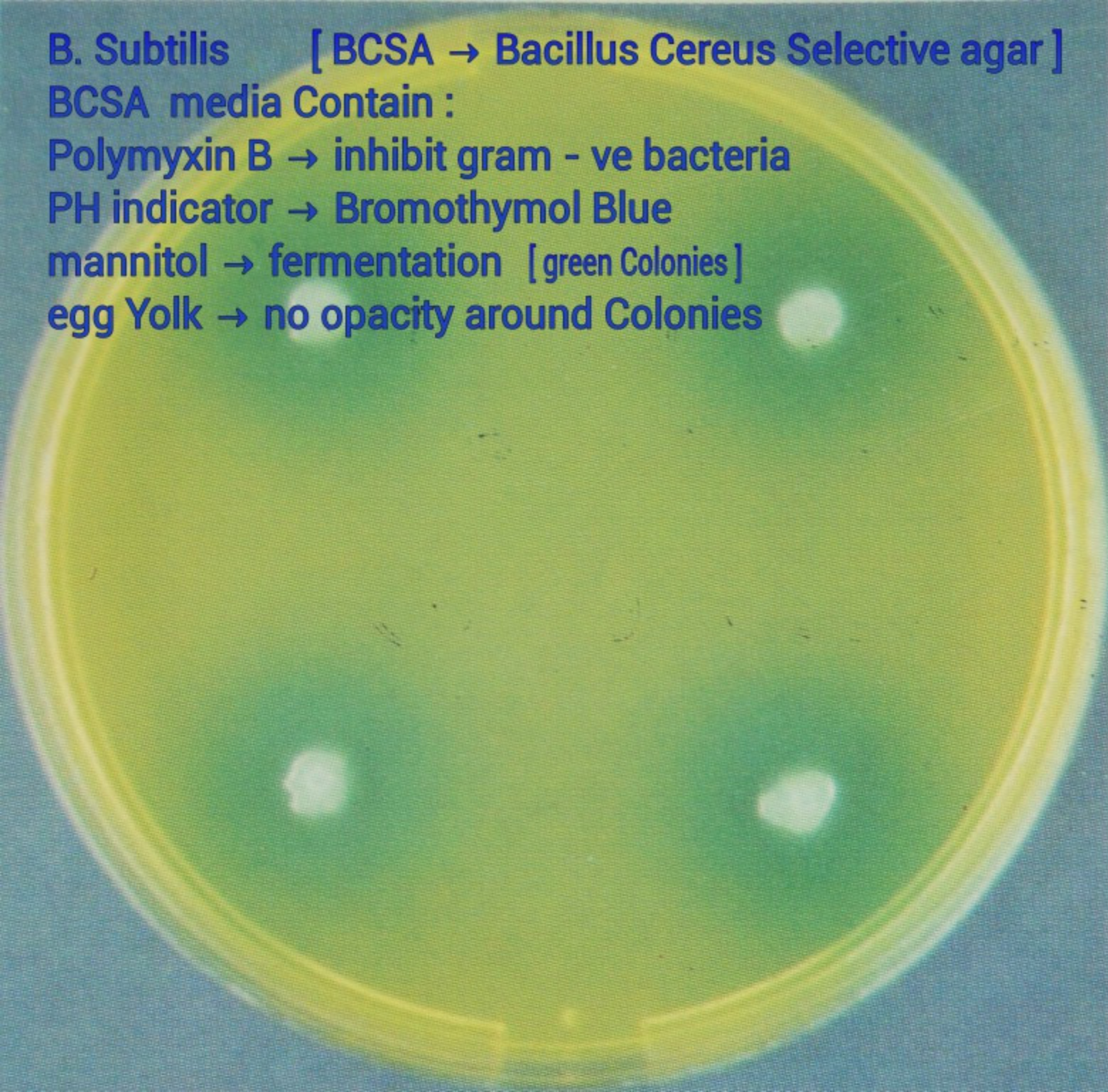
BCSA media Contain :

Polymyxin B → inhibit gram - ve bacteria

PH indicator → Bromothymol Blue

mannitol → fermentation [green Colonies]

egg Yolk → no opacity around Colonies



[*C. perfringens* On Blood agar]

Colonies Surrounded by Two Zones of haemolysis

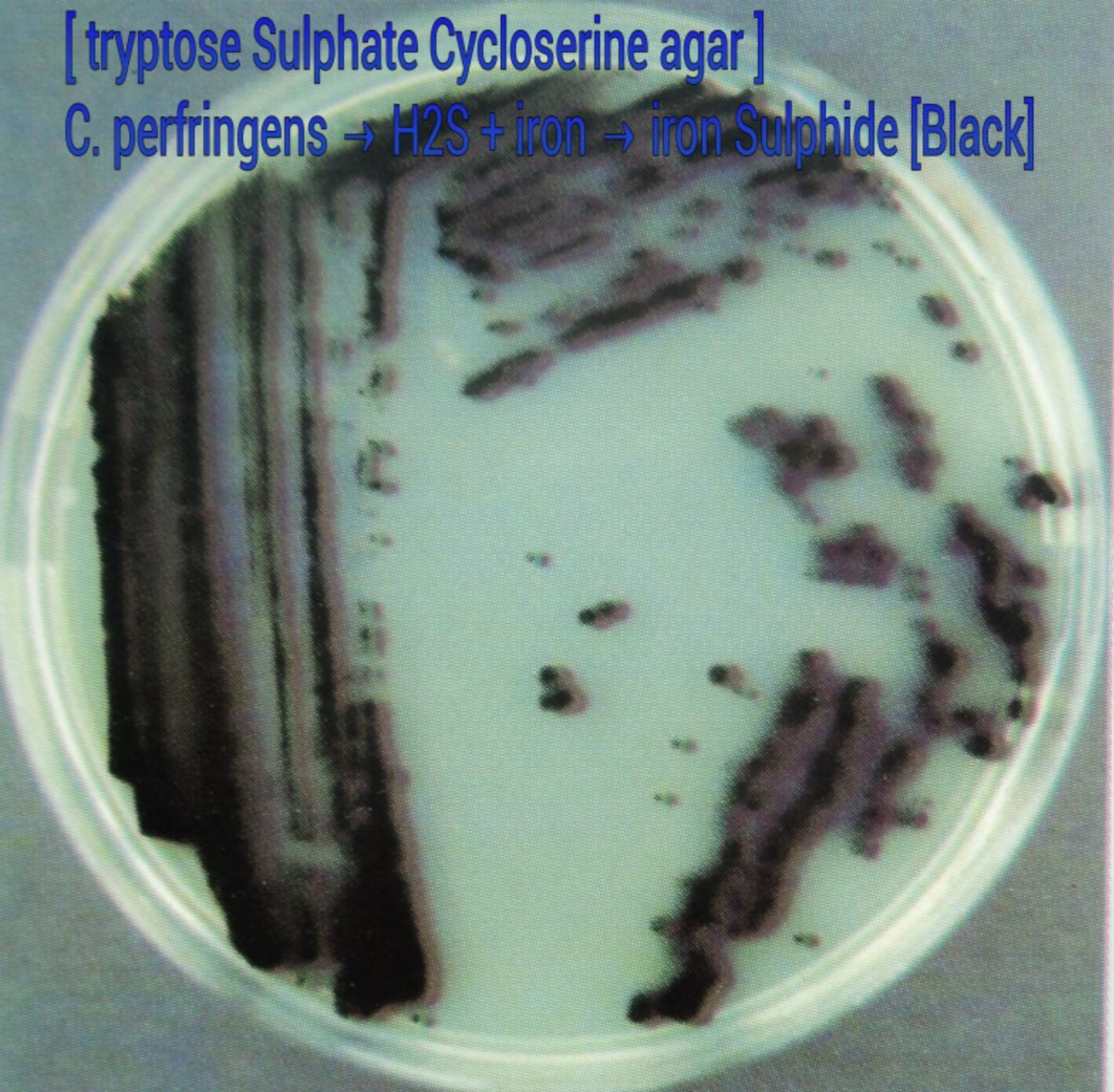
inner → Complete haemolysis due to Theta toxin release

outer → partial haemolysis due to α - Toxin release



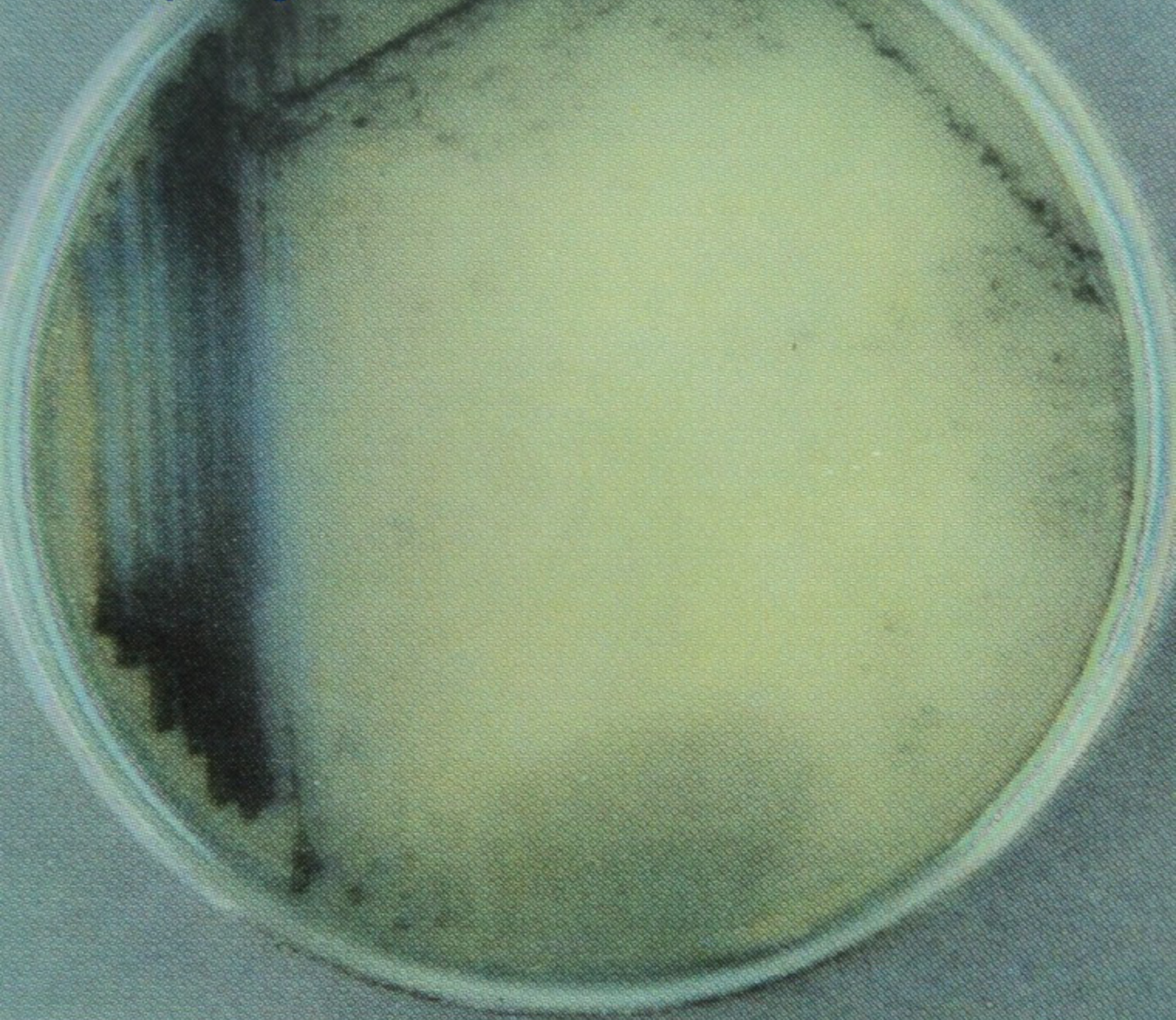
[tryptose Sulphate Cycloserine agar]

C. perfringens → H_2S + iron → iron Sulphide [Black]



[tryptose Sulphate Cycloserine agar]

C. Sporogens → NO H₂S → No Black Colour



[Oxford agar for listeria SPP]

listeria hydrolyse aesculin+ ferrous ions → Black Zones
under and around Colonies



[Palcam agar for listeria SPP]

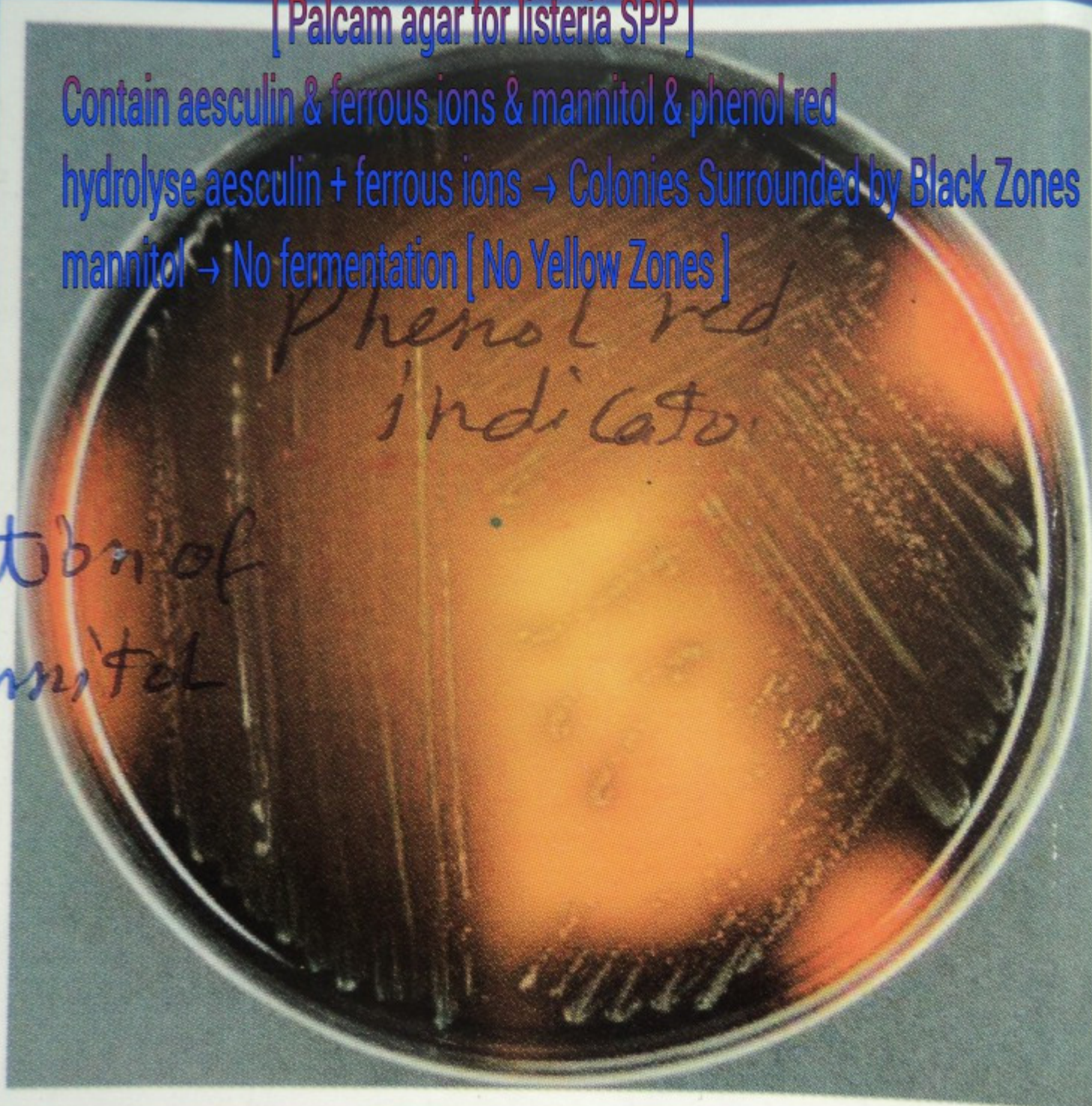
Contain aesculin & ferrous ions & mannitol & phenol red

hydrolyse aesculin + ferrous ions → Colonies Surrounded by Black Zones

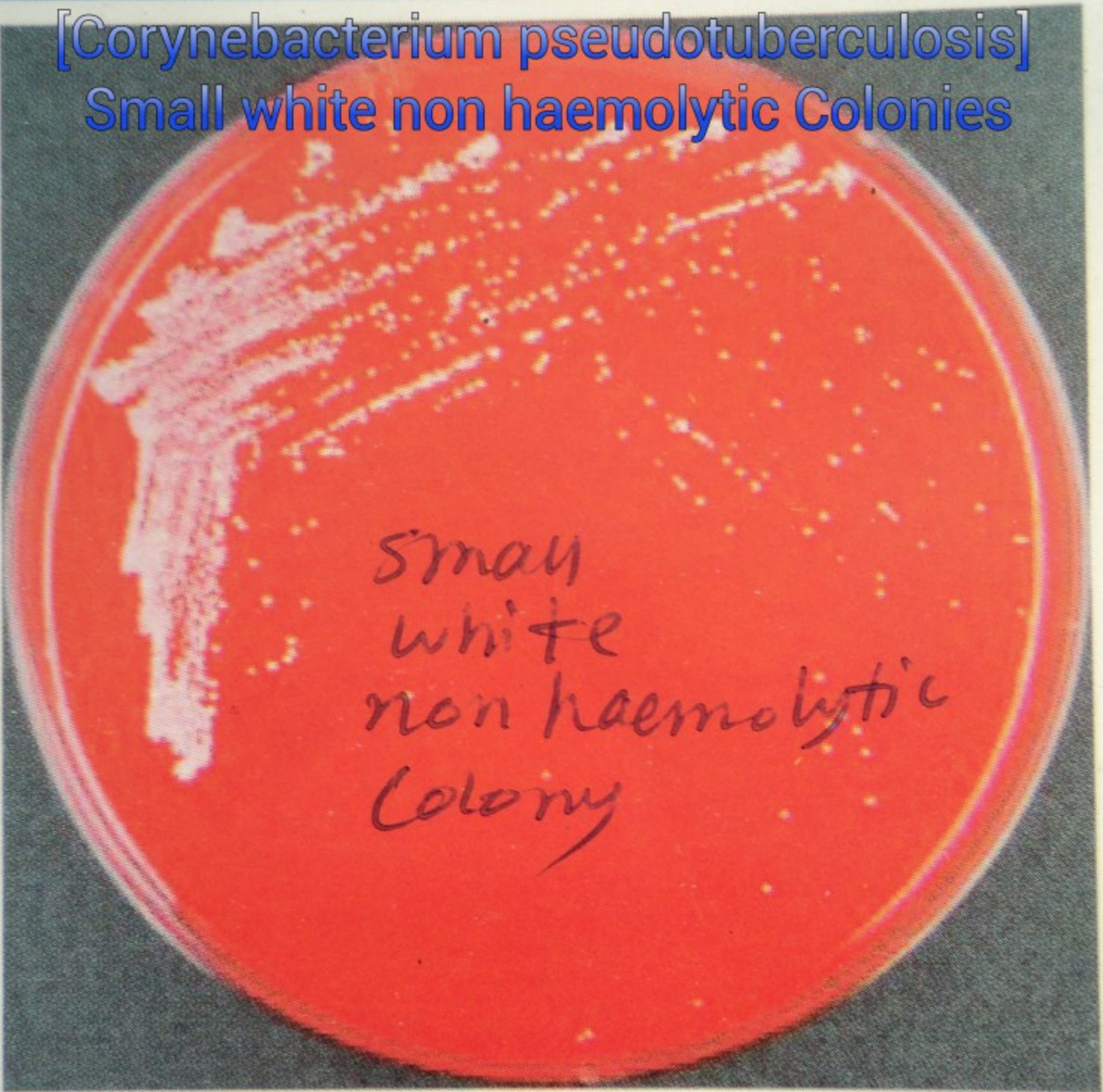
mannitol → No fermentation [No Yellow Zones]

attn of
mannitol

Phenol red
indicator

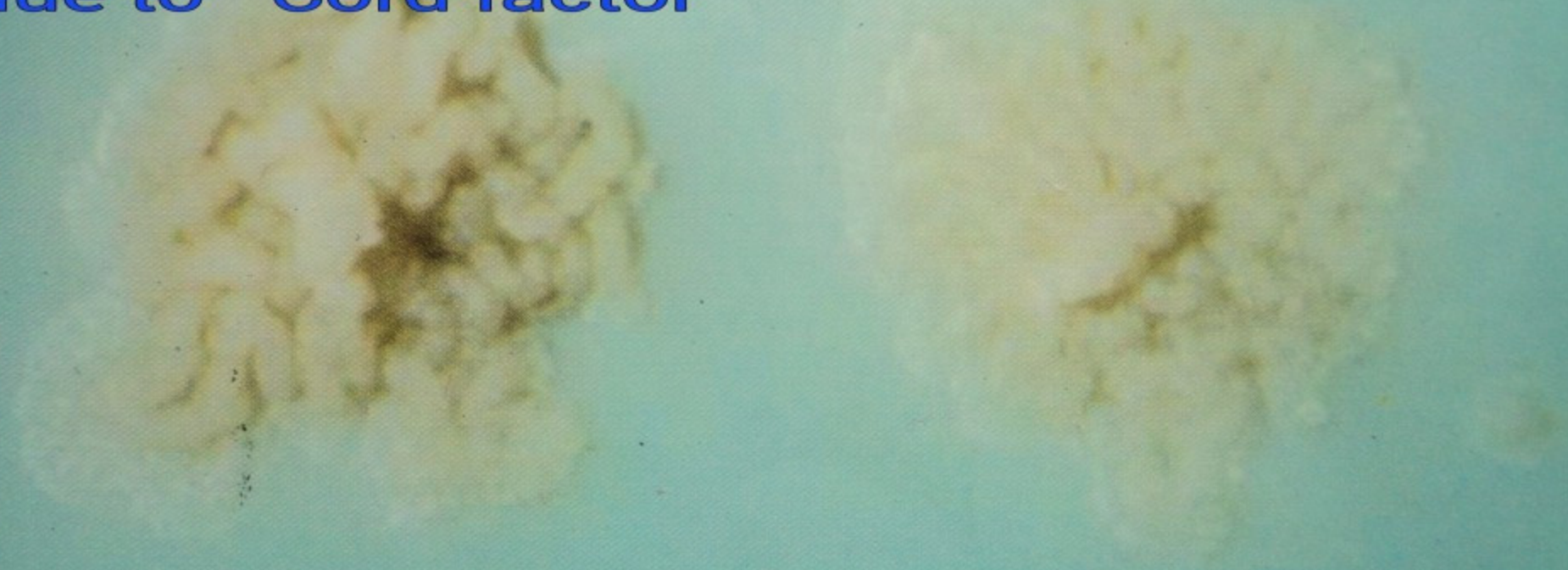


[*Corynebacterium pseudotuberculosis*]
Small white non haemolytic Colonies

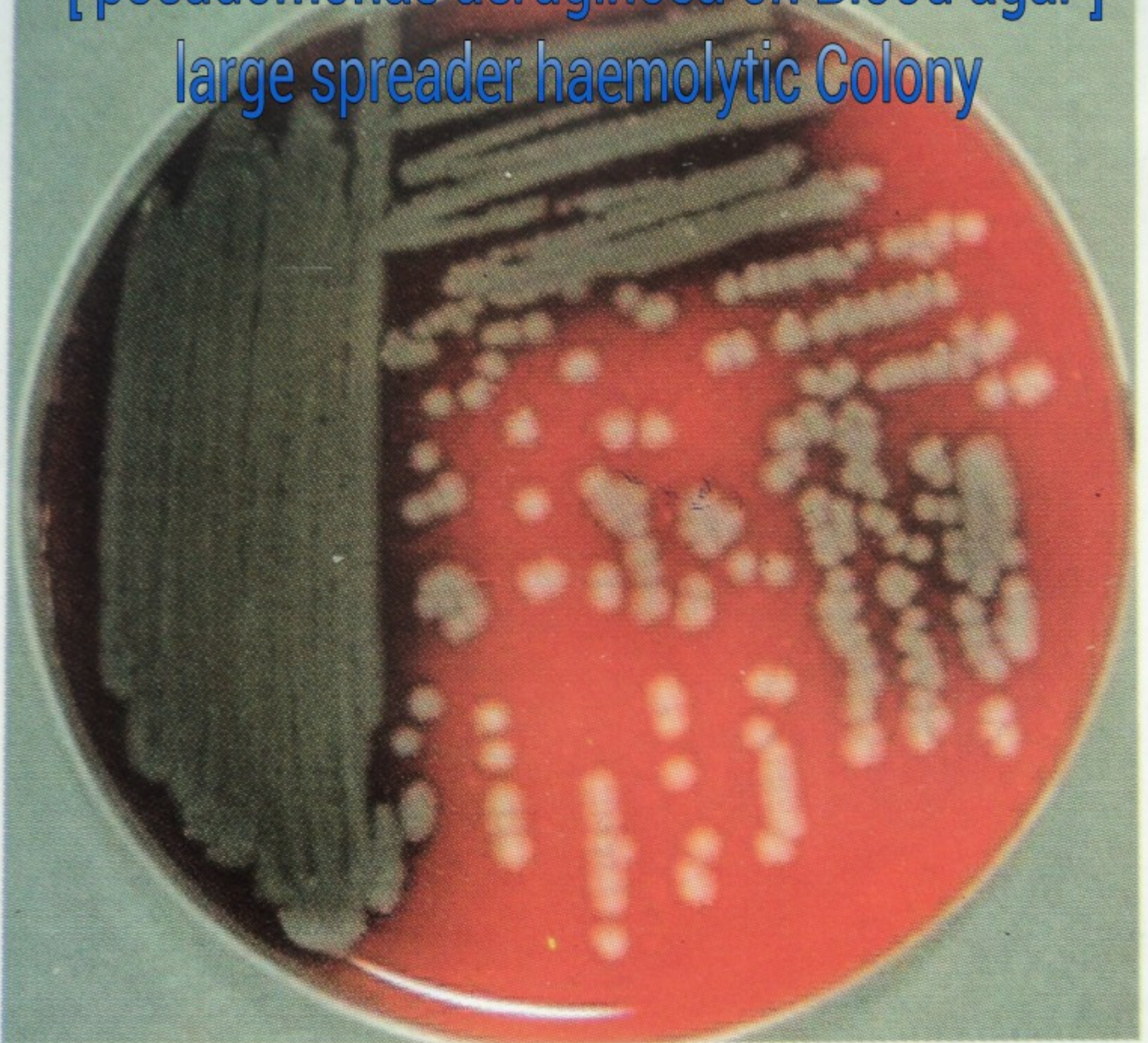
A photograph of a petri dish containing a red agar medium. Numerous small, white, non-haemolytic bacterial colonies are visible, scattered across the surface. A larger, more confluent area of colonies is visible on the left side of the dish. Handwritten text in brown ink is present in the lower right quadrant of the dish.

Small
white
non haemolytic
Colonies

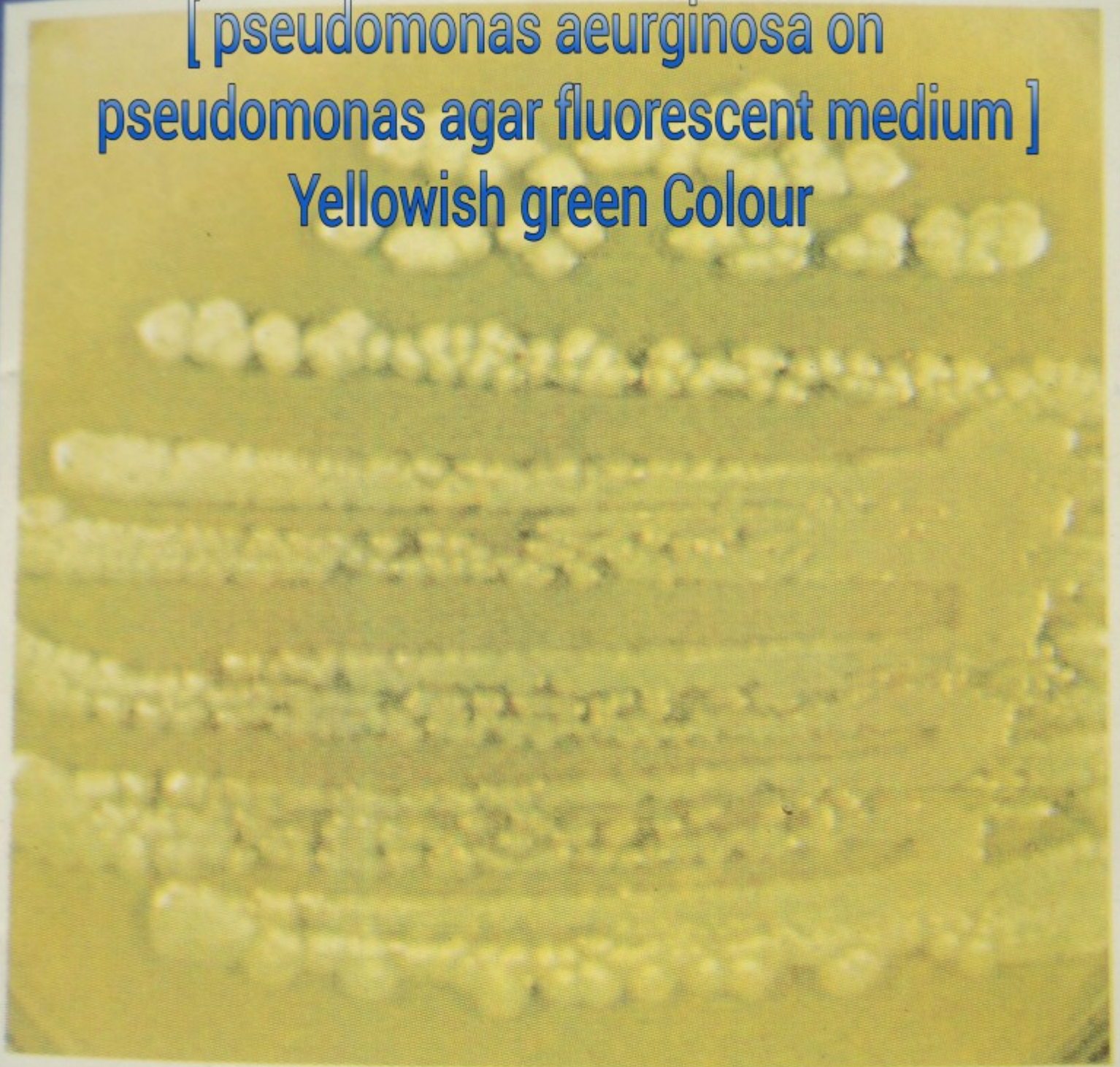
[*Mycobacterium tuberculosis* Colonies
On Lowenstein Jensen medium]
Dry , luxuriant , heavy growth [eugenic]
yellowish Colony with rough Surface
due to Cord factor



[*Pseudomonas aeruginosa* on Blood agar]
large spreader haemolytic Colony



[*Pseudomonas aeruginosa* on
Pseudomonas agar fluorescent medium]
Yellowish green Colour





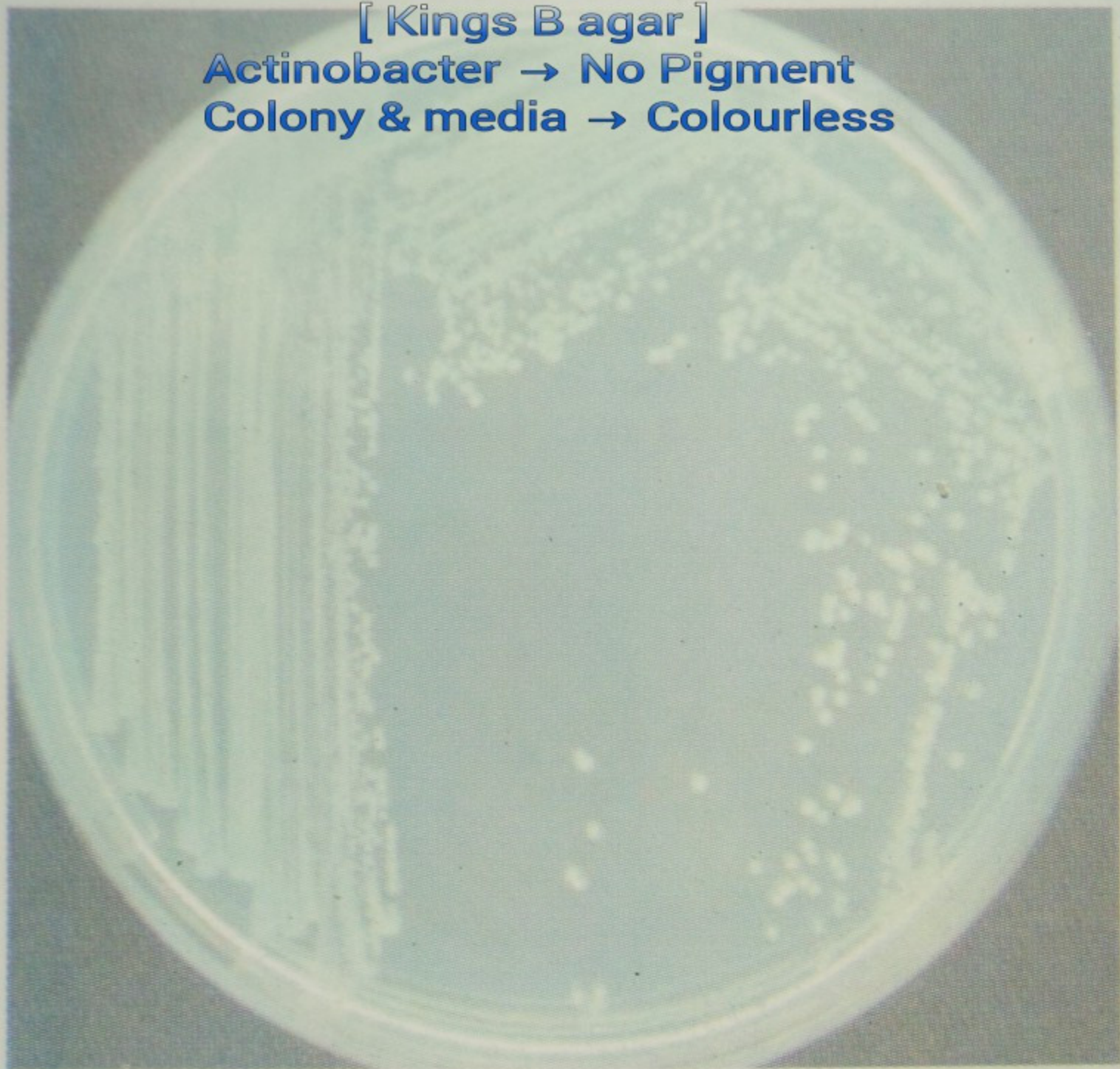
[*Pseudomonas aeruginosa* on
pseudomonas agar pyocyanin medium]
greenish blue Colour

A photograph of a petri dish containing a bacterial culture on King's B agar. The agar surface is a uniform yellowish-green color, indicating the presence of pyoverdine produced by the bacteria. The petri dish is circular and the agar is spread across its entire surface.

[kings B agar]

pseudomonas fluorescense → Pyoverdin
media & Colony → Yellowish green

[Kings B agar]
Actinobacter → No Pigment
Colony & media → Colourless

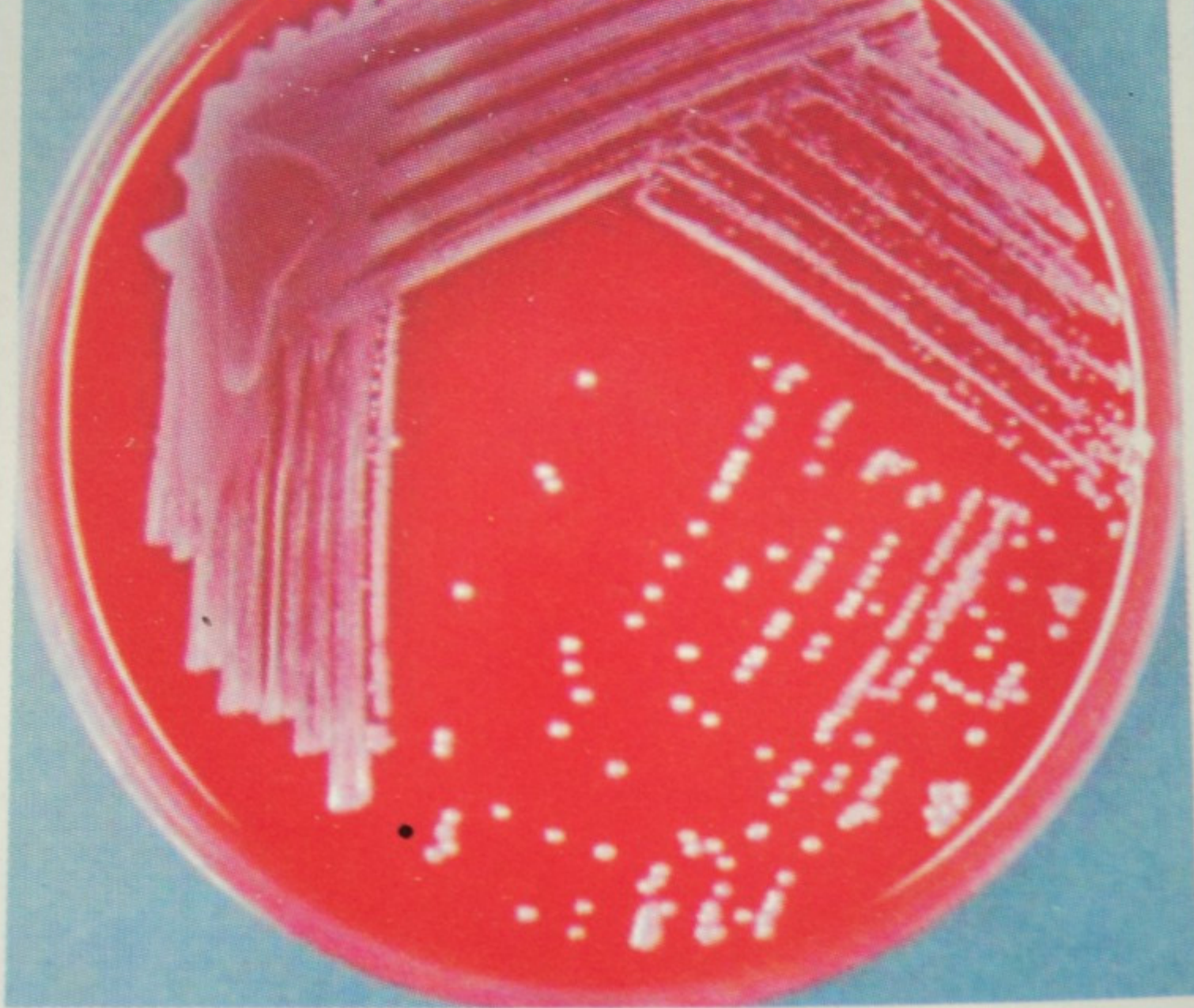


[Brucella]

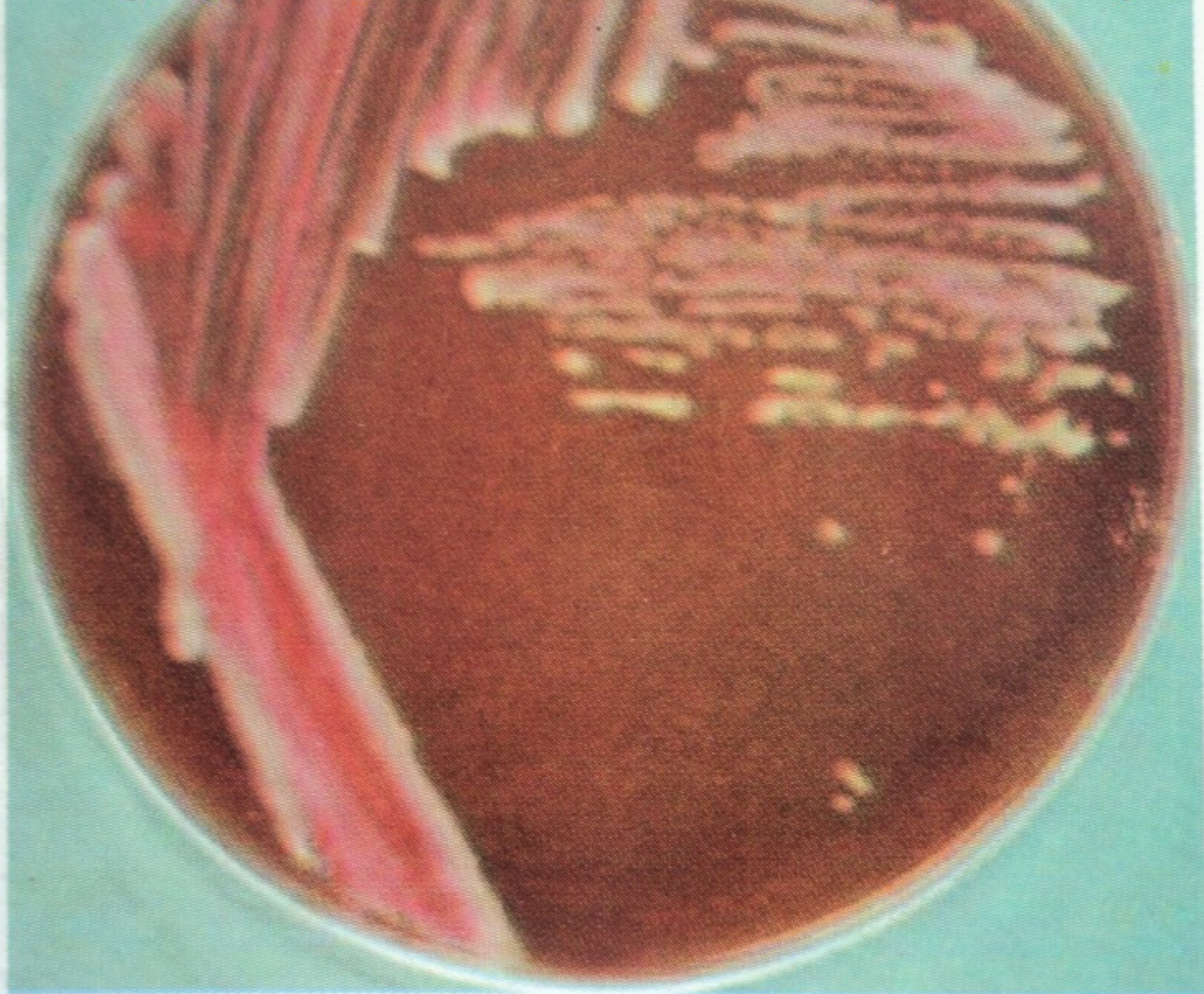
non haemolytic Small Colony on Blood agar

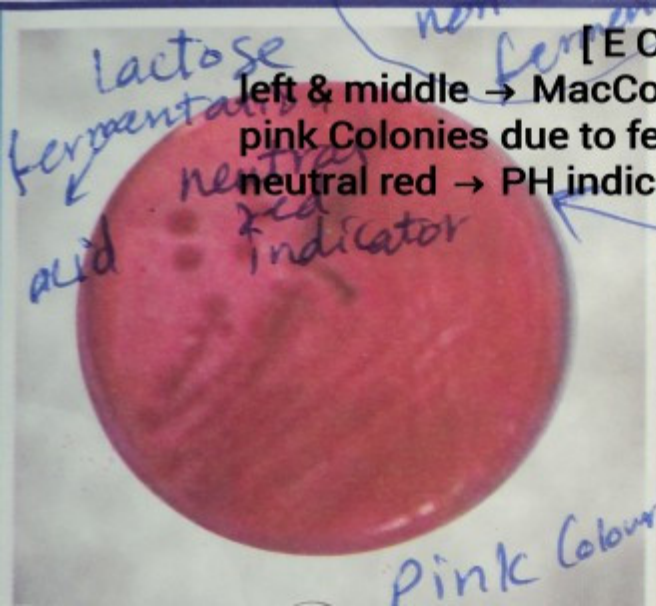


[Bordetella bronchiseptica]
Non haemolytic Small Colony on Blood agar

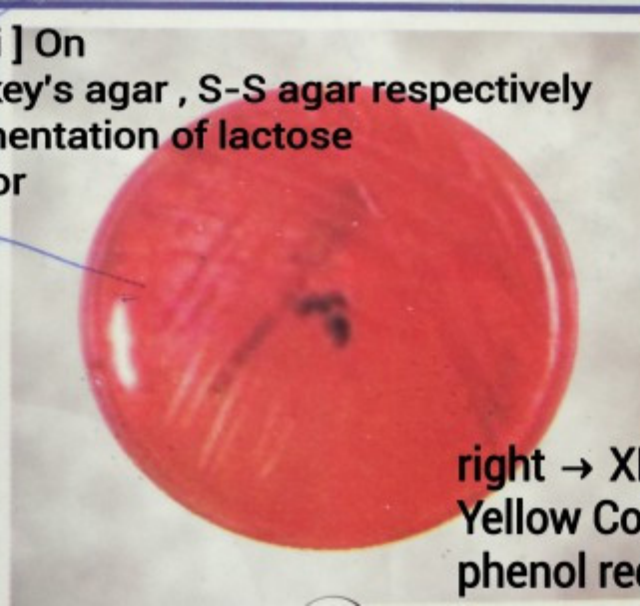


[*Campylobacter jejuni*]
grayish spreader non haemolytic Colony

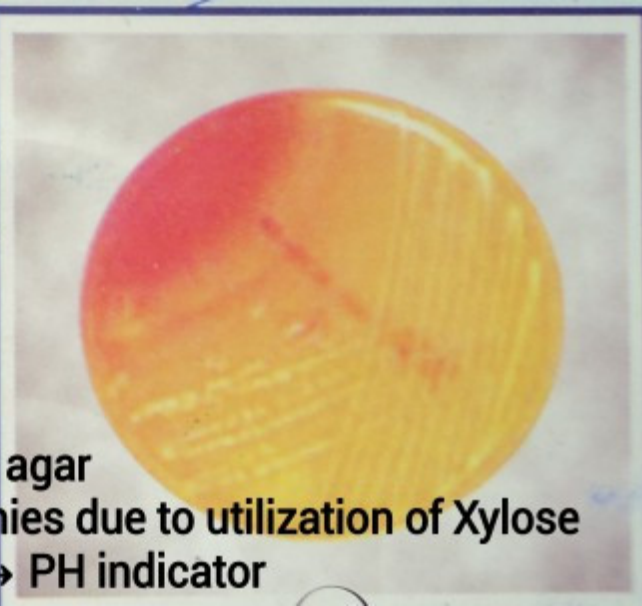




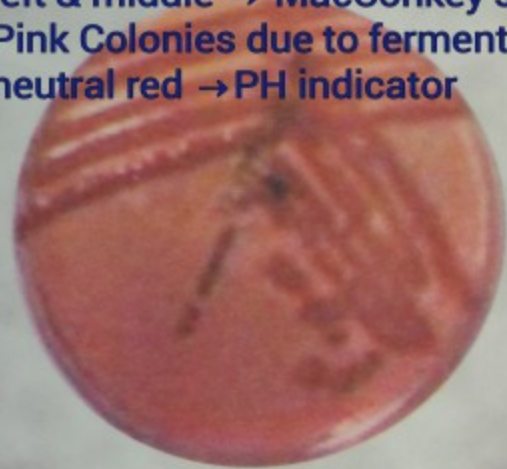
[E Coli] On
left & middle → MacConkey's agar , S-S agar respectively
pink Colonies due to fermentation of lactose
neutral red → PH indicator



right → XLD agar
Yellow Colonies due to utilization of Xylose
phenol red → PH indicator



[Klebsiella] On
left & middle → MacConkey's agar , S-S agar respectively
Pink Colonies due to fermentation of lactose
neutral red → PH indicator

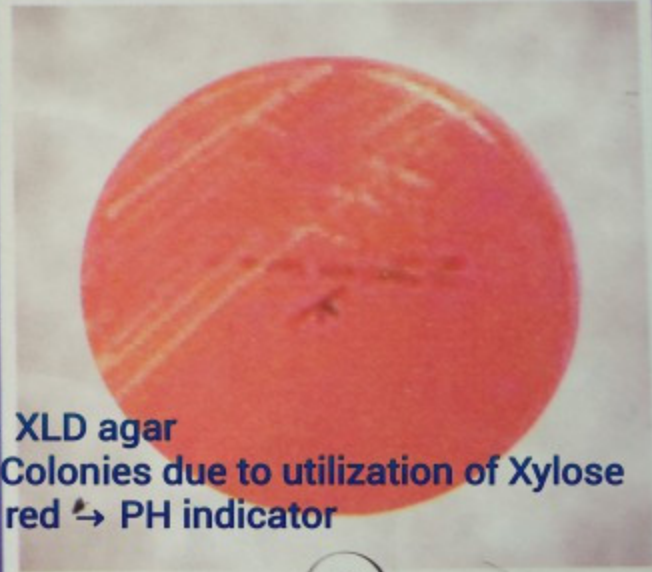


1



2

right → XLD agar
Yellow Colonies due to utilization of Xylose
phenol red → PH indicator



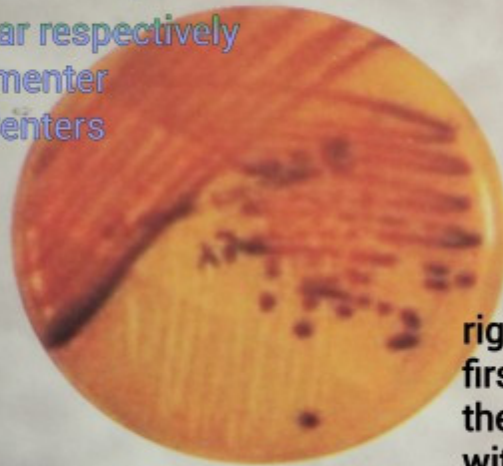
3

[Salmonella] On

left & middle → MacConkey's agar , S-S agar respectively

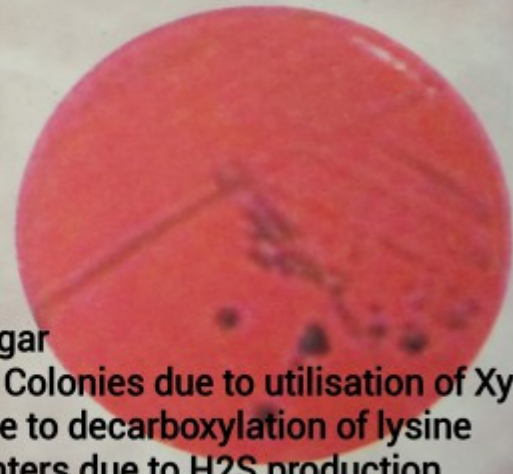
Colourless Colonies due to non lactose fermenter

Middle → H_2S production + iron → Black Centers



right → XLD agar

first → Yellow Colonies due to utilisation of Xylose
then → red due to decarboxylation of lysine
with black Centers due to H_2S production



[E Coli on Eosin Methylene Blue] EMB agar
Sucrose + Lactose → fermentation and formation of
Methylene Blue eosinate Complex [metallic Sheen]



[Klebsiella on Eosin Methylene Blue] EMB agar
Colourless or light Purple Colonies
no Sucrose and lactose

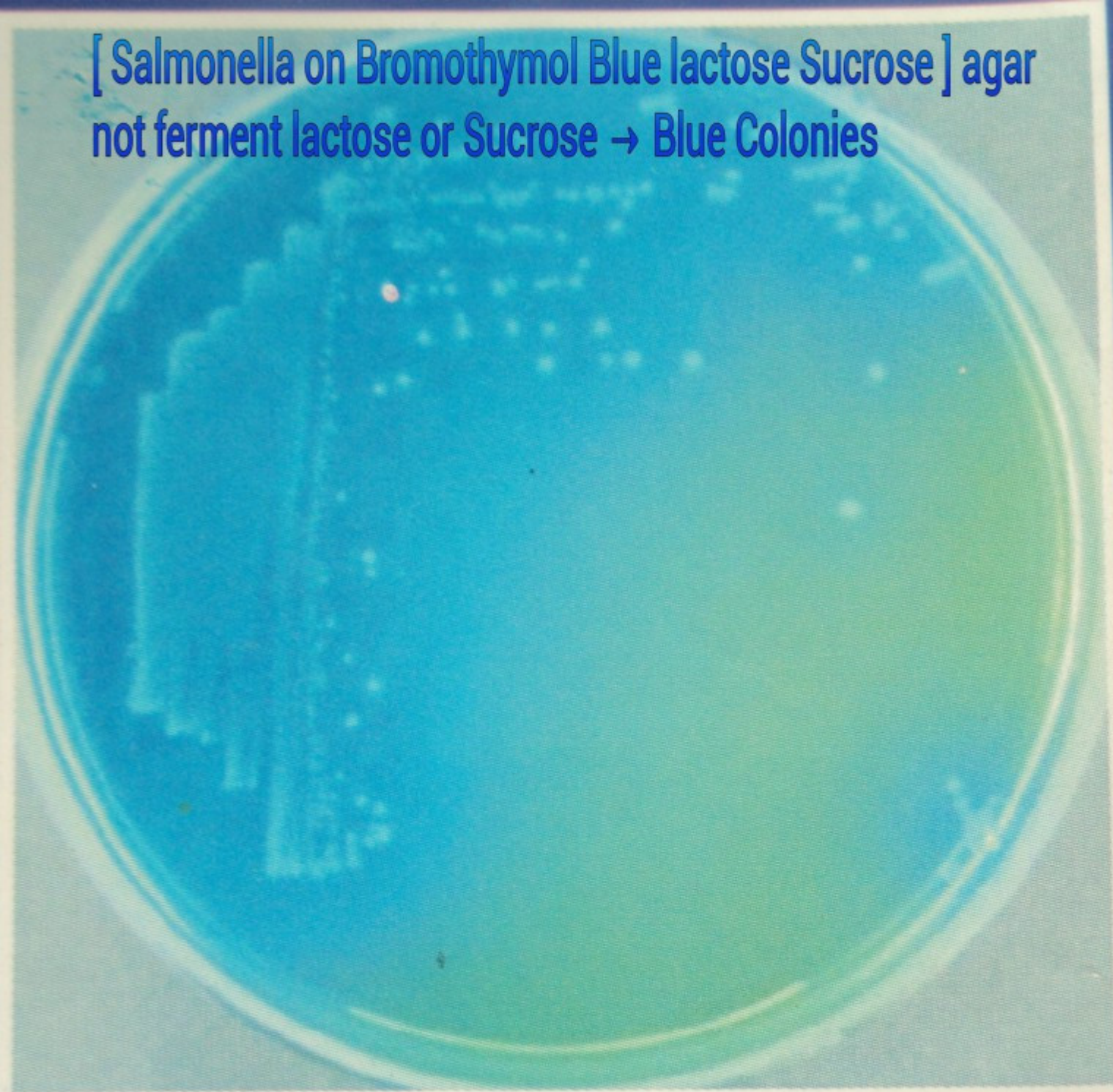


[E Coli on Bromothymol Blue lactose Sucrose] agar
ferment lactose and / or Sucrose → Yellow Colonies

at 16h

A photograph of a petri dish containing a bacterial culture. The agar is a pale yellow color, and there are numerous small, yellow, circular colonies scattered across the surface. The colonies are more densely packed in some areas, particularly towards the bottom right. The petri dish is set against a dark blue background.

[Salmonella on Bromothymol Blue lactose Sucrose] agar
not ferment lactose or Sucrose → Blue Colonies



BPLS [Brilliant-green phenol-red lactose Sucrose] agar

Salmonella → red Colonies due to no fermentation of Lactose or sucrose

not auto



BPLS [Brilliant-green phenol-red lactose Sucrose] agar
fermentation of lactose and / or Sucrose → Acid
→ low PH → Yellow Colonies

E Coli

at 16m



[*Proteus mirabilis*]
Swarming on Blood agar due to
active Motile Microorganism





[*Pasteurella Multocida*]
non haemolytic white Colony
on Blood agar

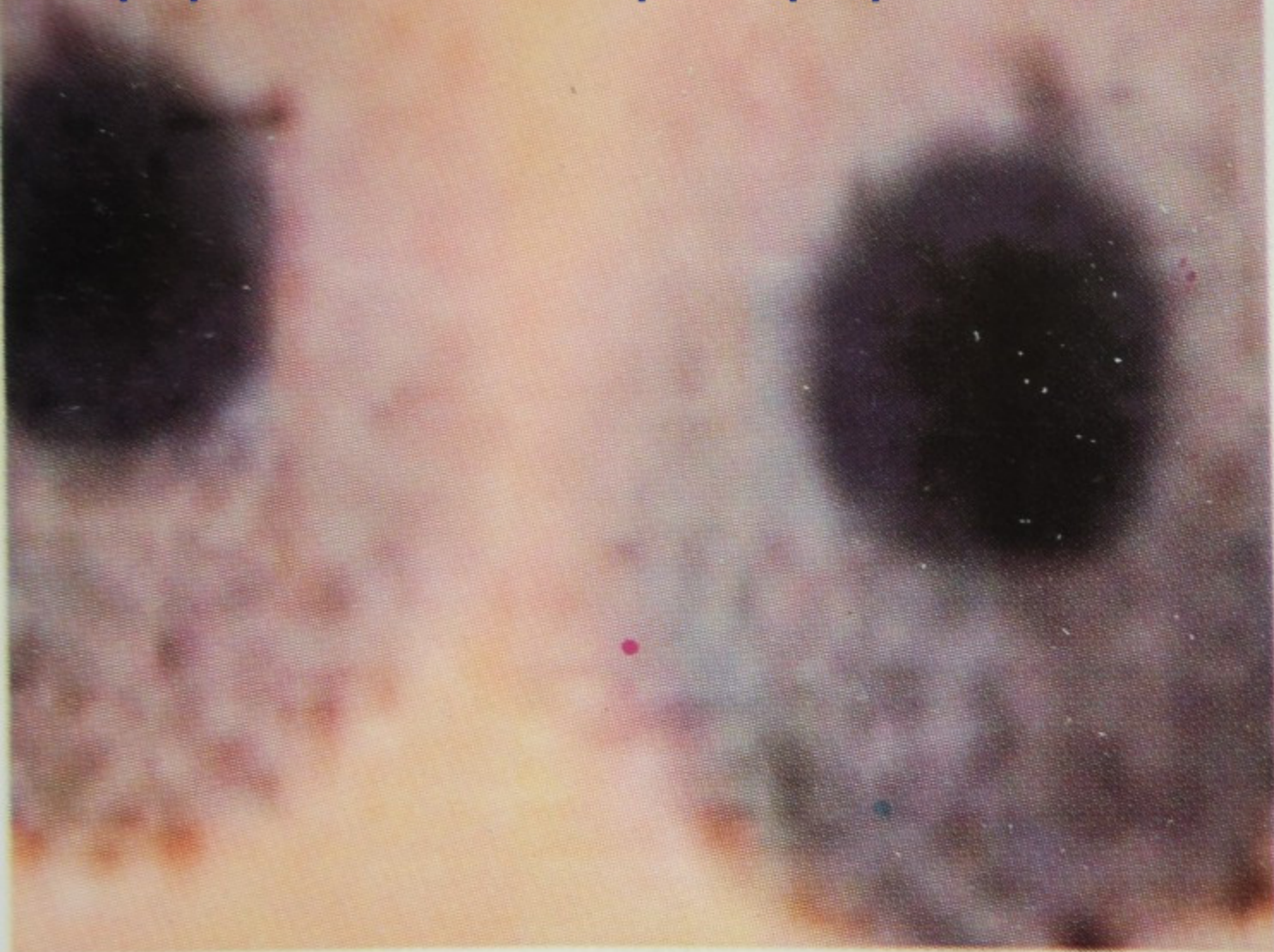
A petri dish containing a bacterial culture on blood agar. The medium is a deep red color. There are several streaks of bacterial growth visible. The growth shows complete (beta) haemolysis, characterized by the formation of large, clear, colorless zones where the red blood cells have been lysed. The text "[Pasteurella haemolytica]" and "B- haemolysis on Blood agar" is overlaid on the bottom right of the image.

[*Pasteurella haemolytica*]
B- haemolysis on Blood agar

[Mycoplasma Micro Colonies]

fried egg Micro Colony

Opaque Center with transparent peripheral Zone



[Mycoplasma Micro Colonies]

fried egg Micro Colony

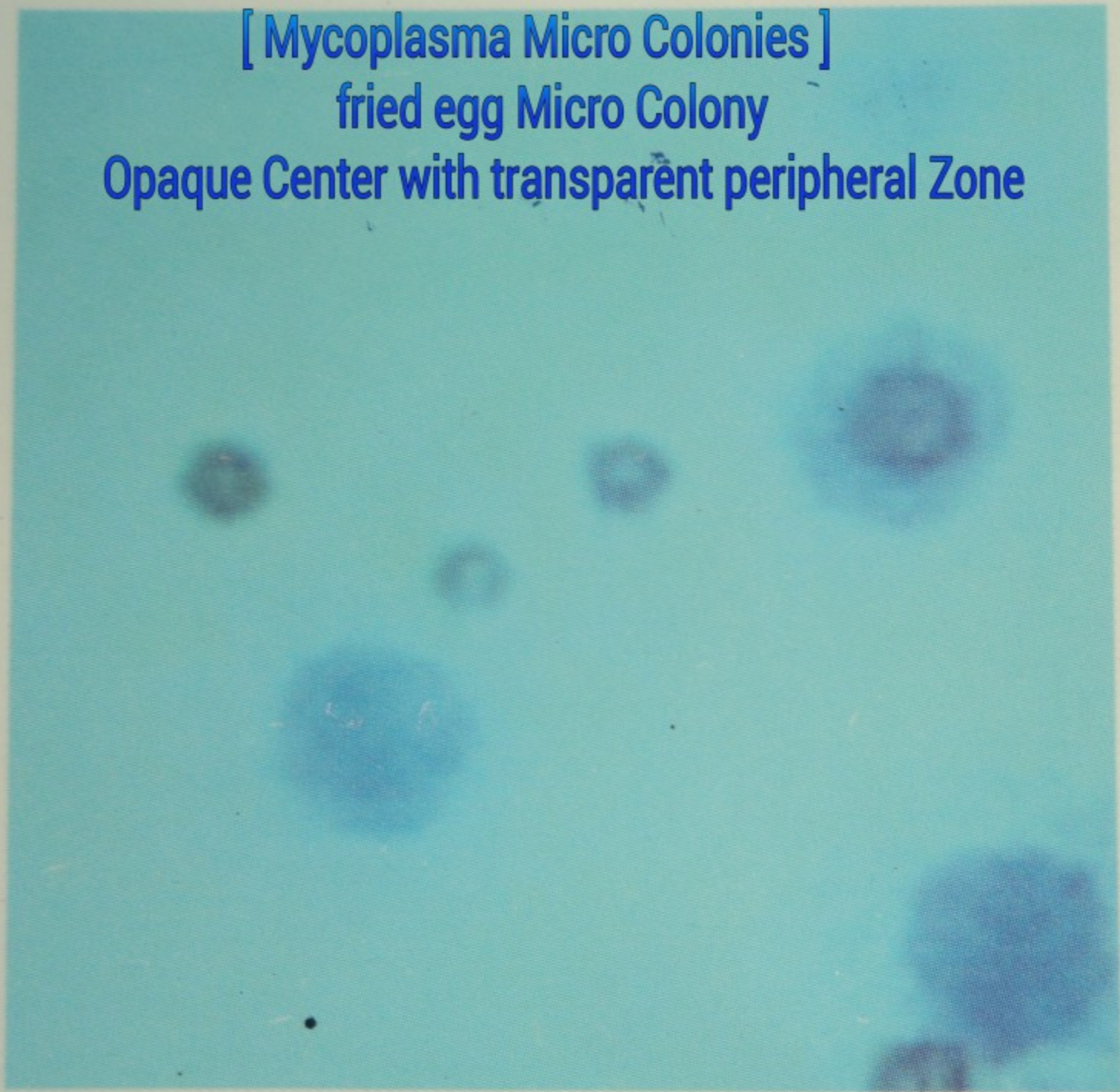
Opaque Center with transparent peripheral Zone



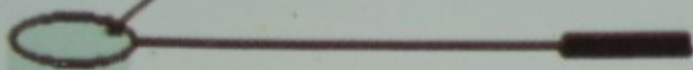
[Mycoplasma Micro Colonies]

fried egg Micro Colony

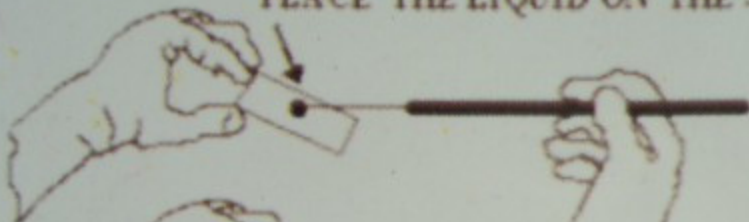
Opaque Center with transparent peripheral Zone



LIQUID CULTURE OR STERILE WATER



PLACE THE LIQUID ON THE SLIDE



ADD THE MICROBES TO THE
LIQUID AND SPREAD OVER
A 1 CM AREA

AIR DRY OR HEAT GENTLY. WHEN DRY
BRIEFLY HEAT FIX THE CELLS TO THE
SLIDE

Preparation of bacterial film

Gram Positive

Gram Negative



Fixation



Crystal violet



Iodine treatment



Decolorization



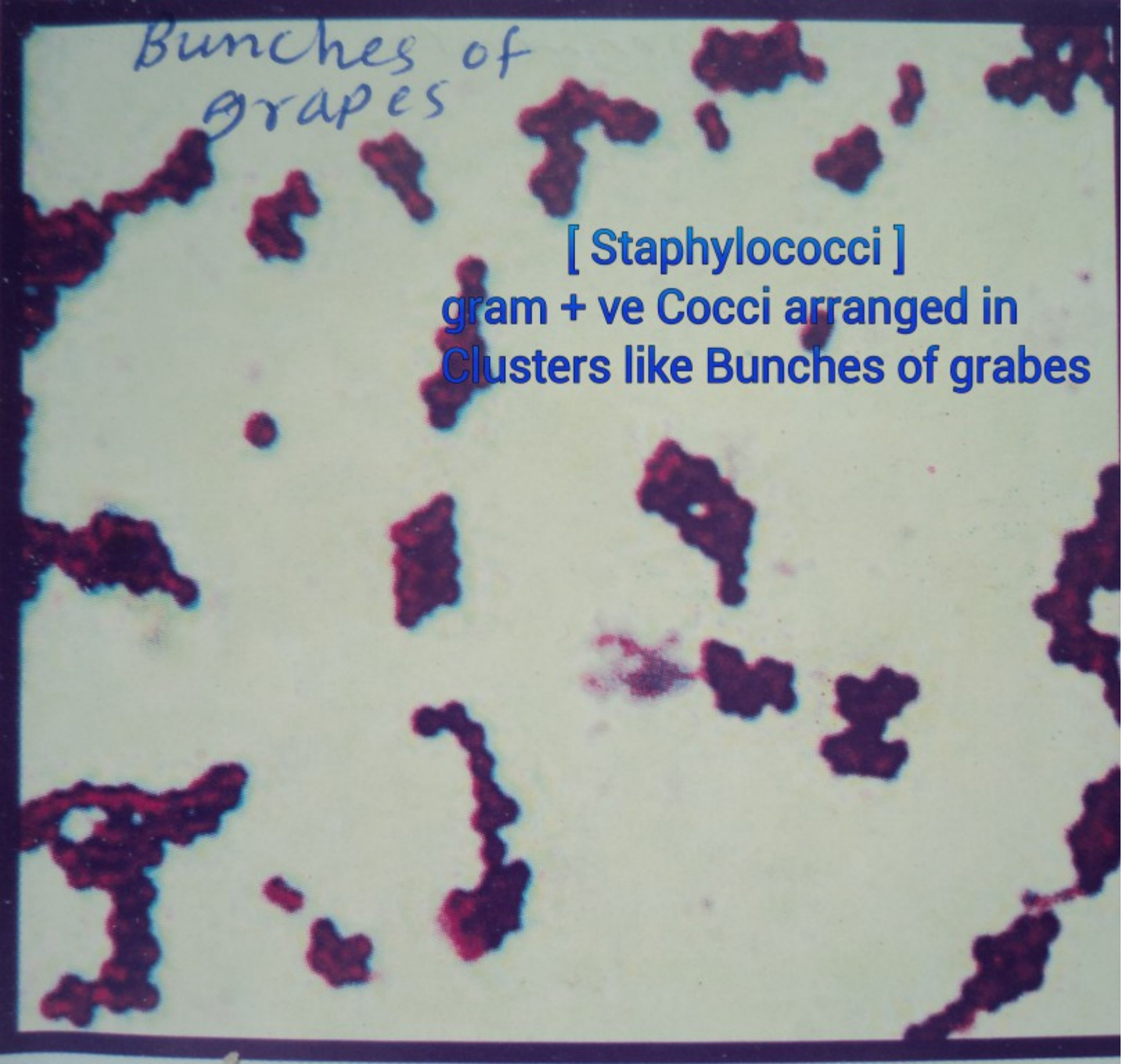
Counter stain
safranin



Gram's stain

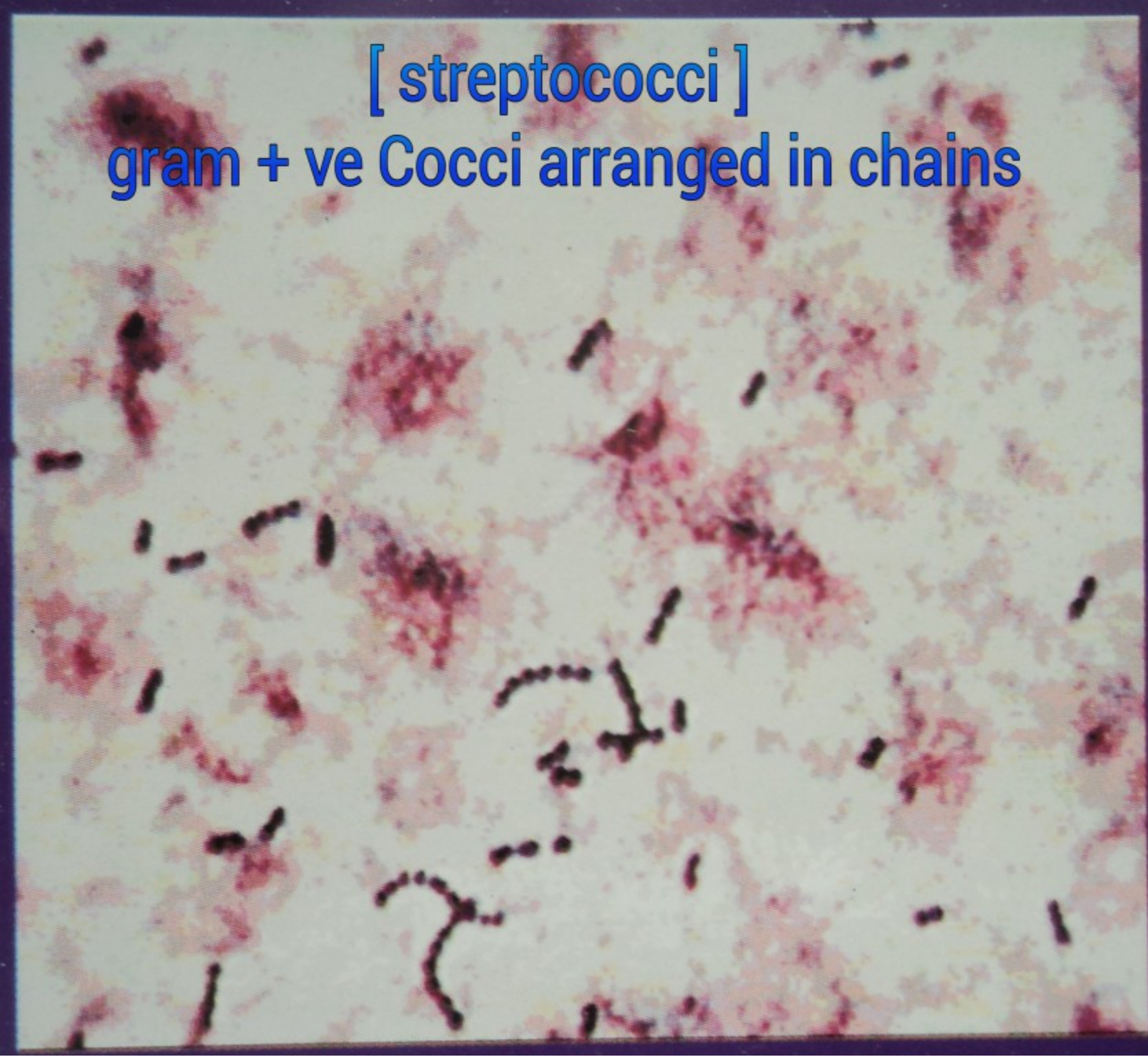
Bunches of
grapes

[Staphylococci]
gram + ve Cocci arranged in
Clusters like Bunches of grapes

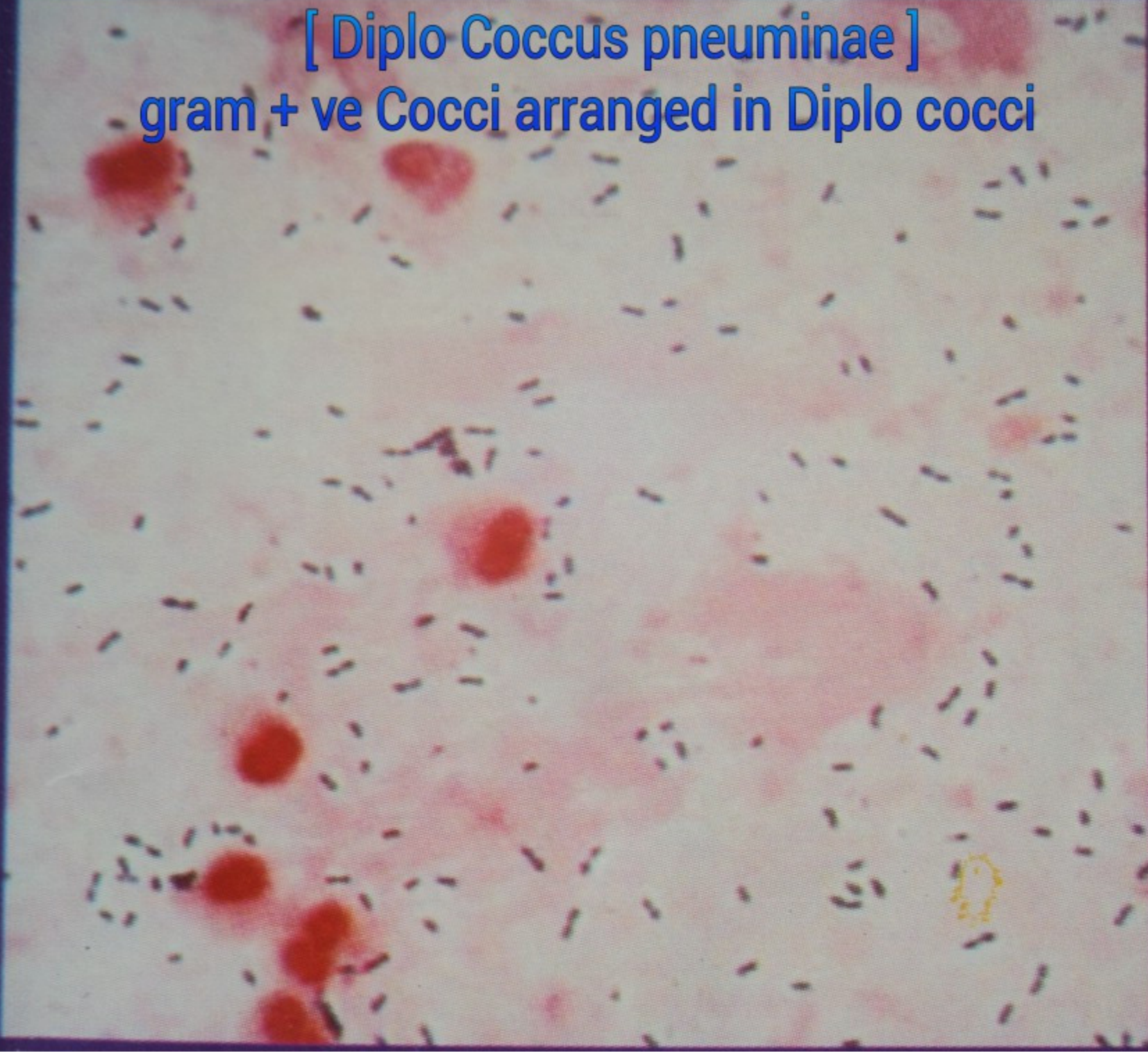


[streptococci]

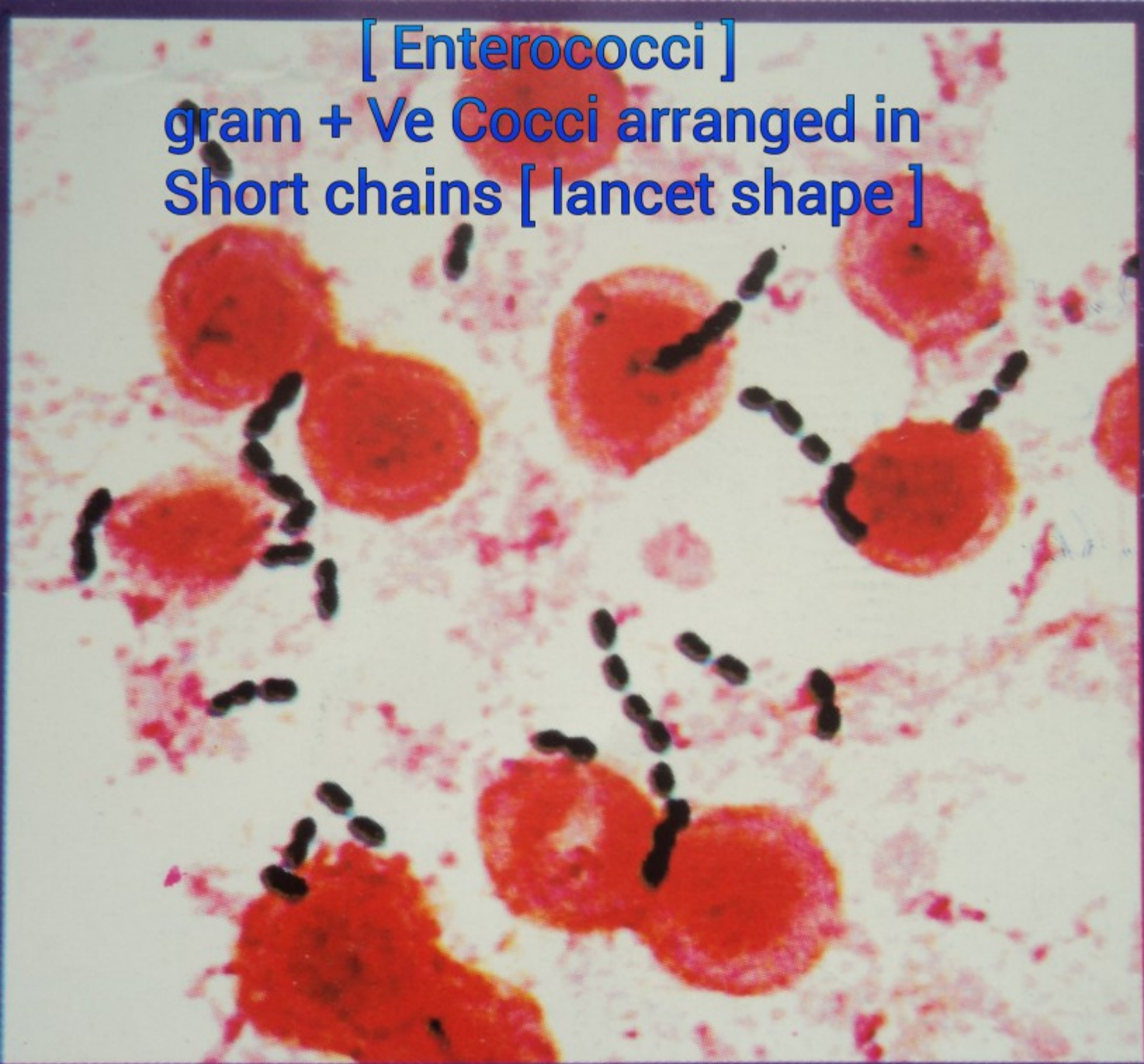
gram + ve Cocci arranged in chains



[*Diplo Coccus pneumoniae*]
gram + ve Cocci arranged in Diplo cocci



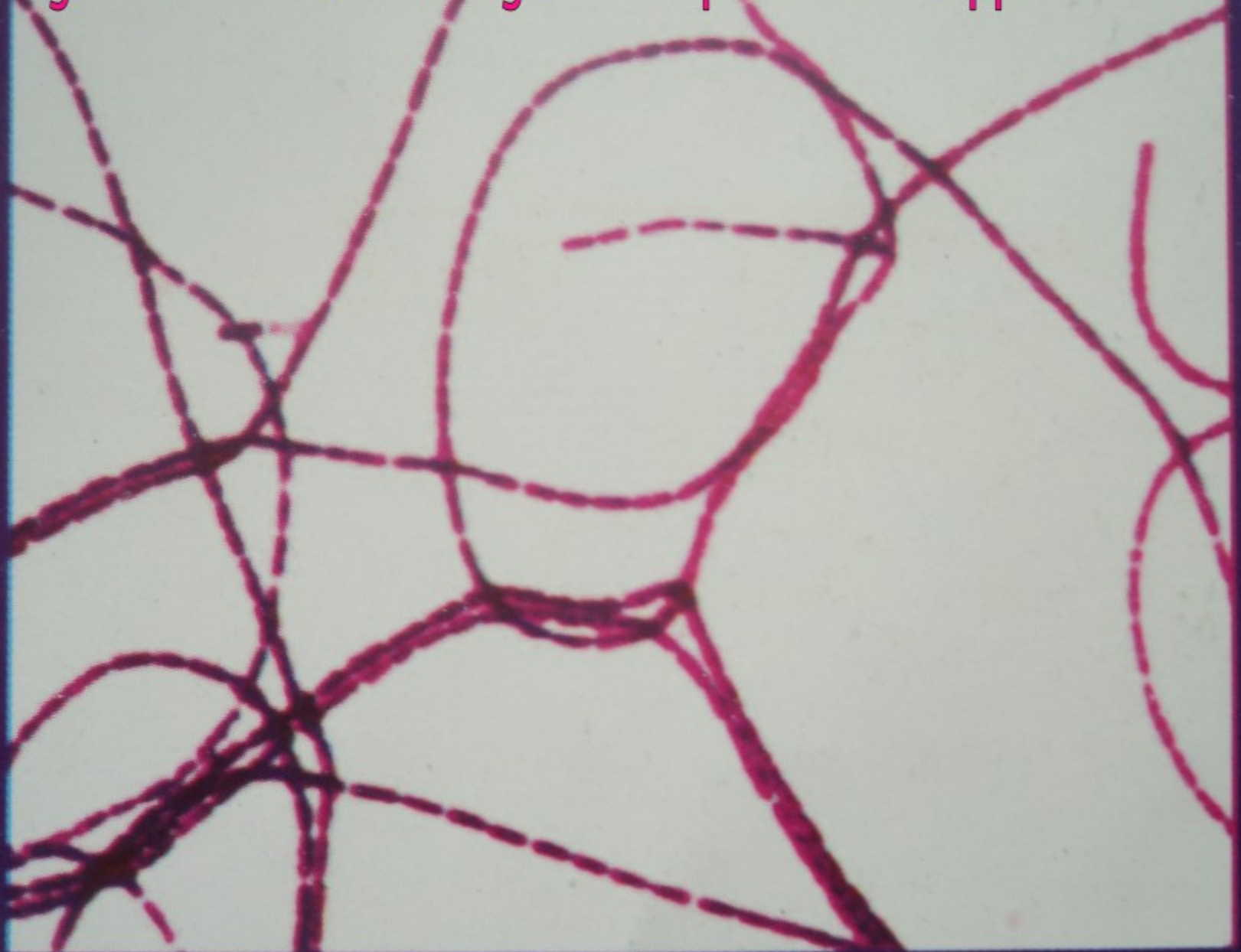
[Enterococci]
gram + Ve Cocci arranged in
Short chains [lancet shape]



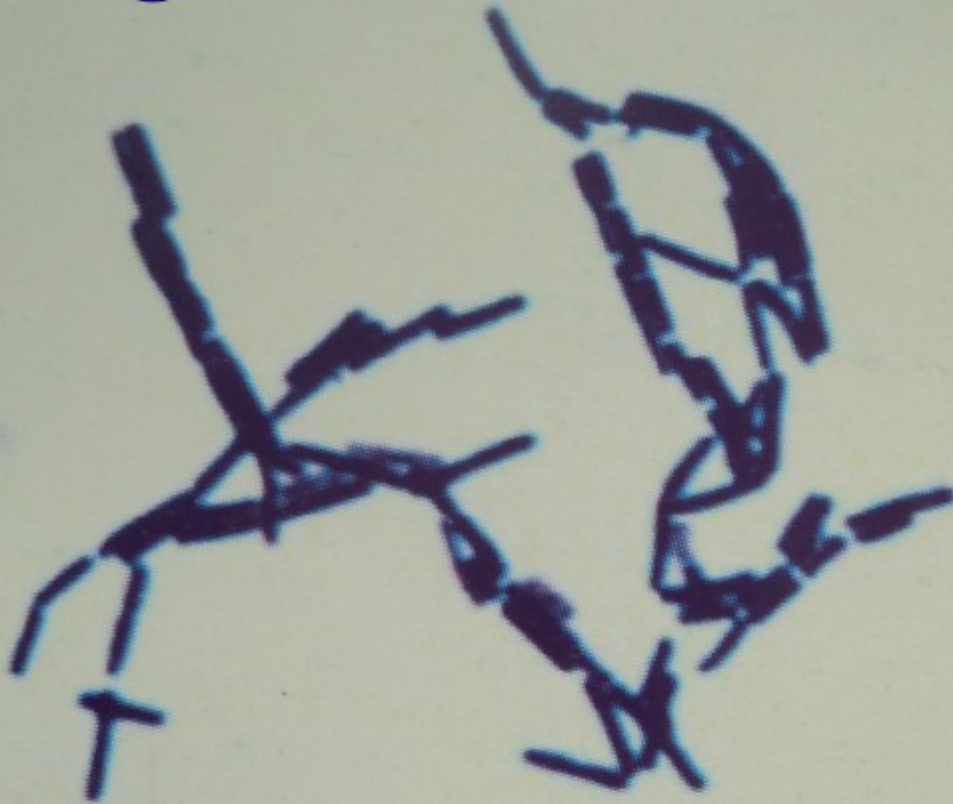
[*B. anthracis* from Culture Containing oxalates]
gram + ve Bacilli arranged in Chains with Central
oval Spore



[*B. anthracis* from Culture Containing Calcium Chloride]
gram + ve Bacilli arranged in Serpentine like appearance



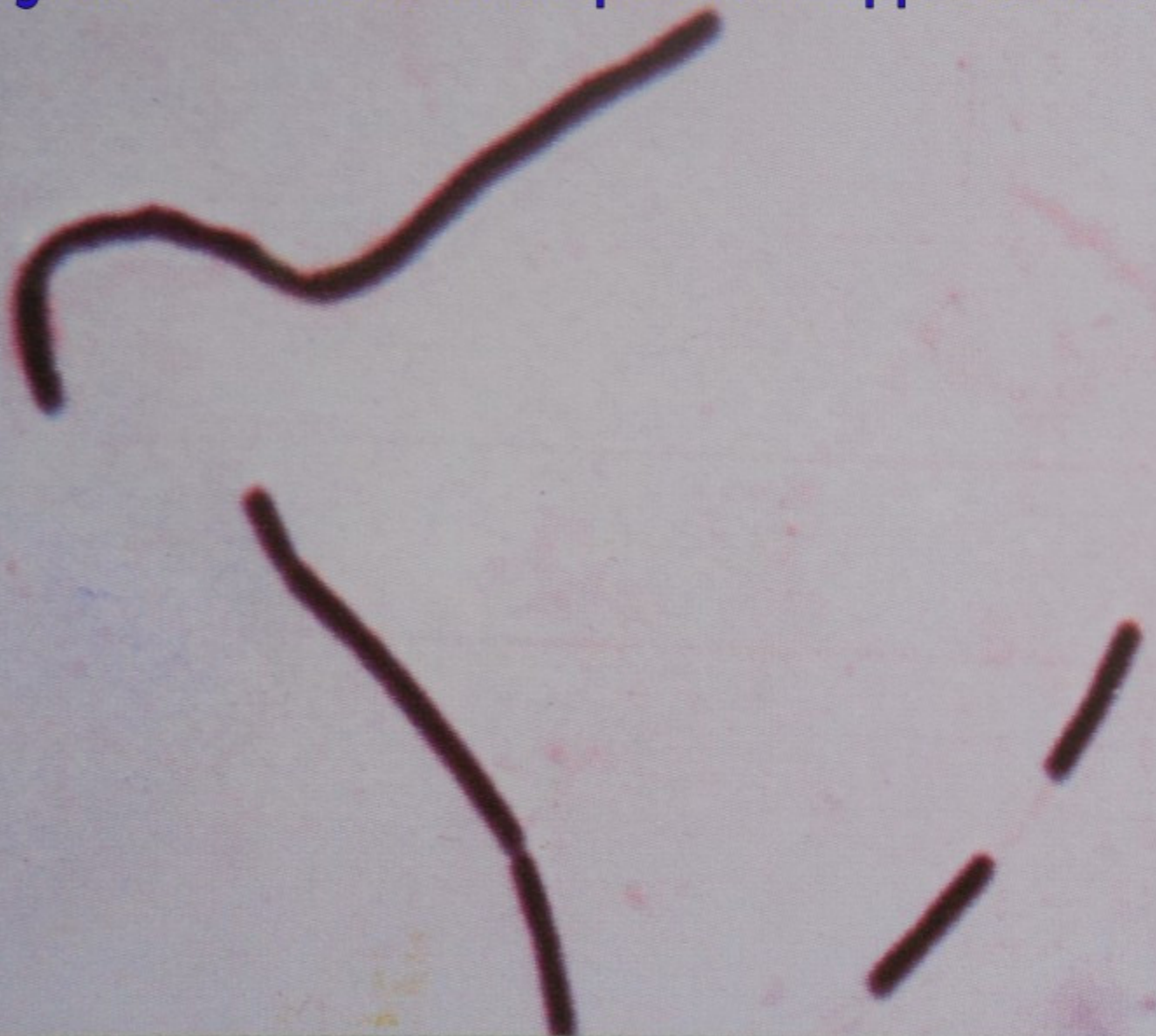
[B. Cereus]
gram + ve Bacilli



[C. tetani]
gram + Ve Bacilli with
drumstick like appearance

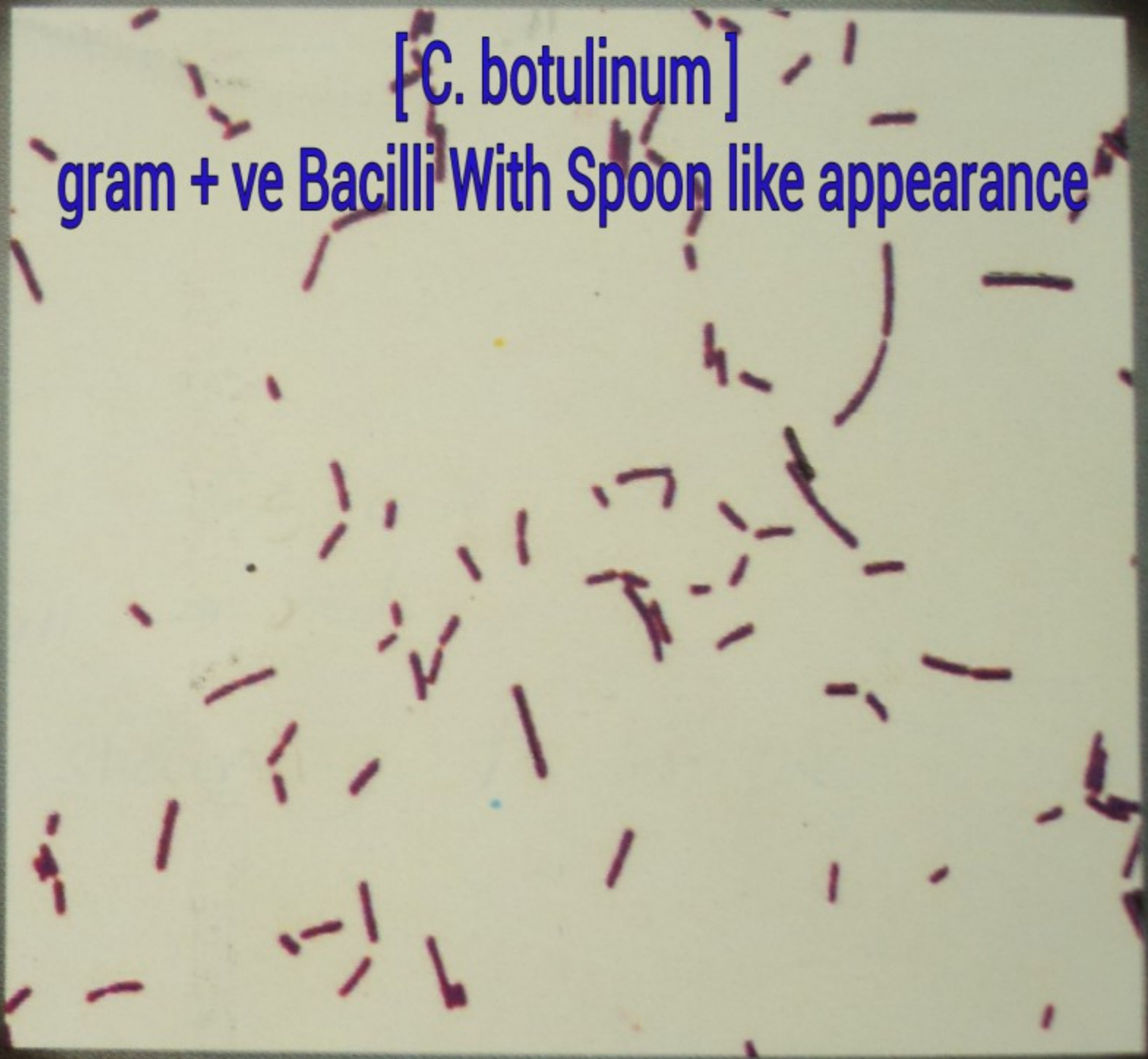


[*C. botulinum*]
gram + ve Bacilli With Spoon like appearance



[*C. botulinum*]

gram + ve Bacilli With Spoon like appearance



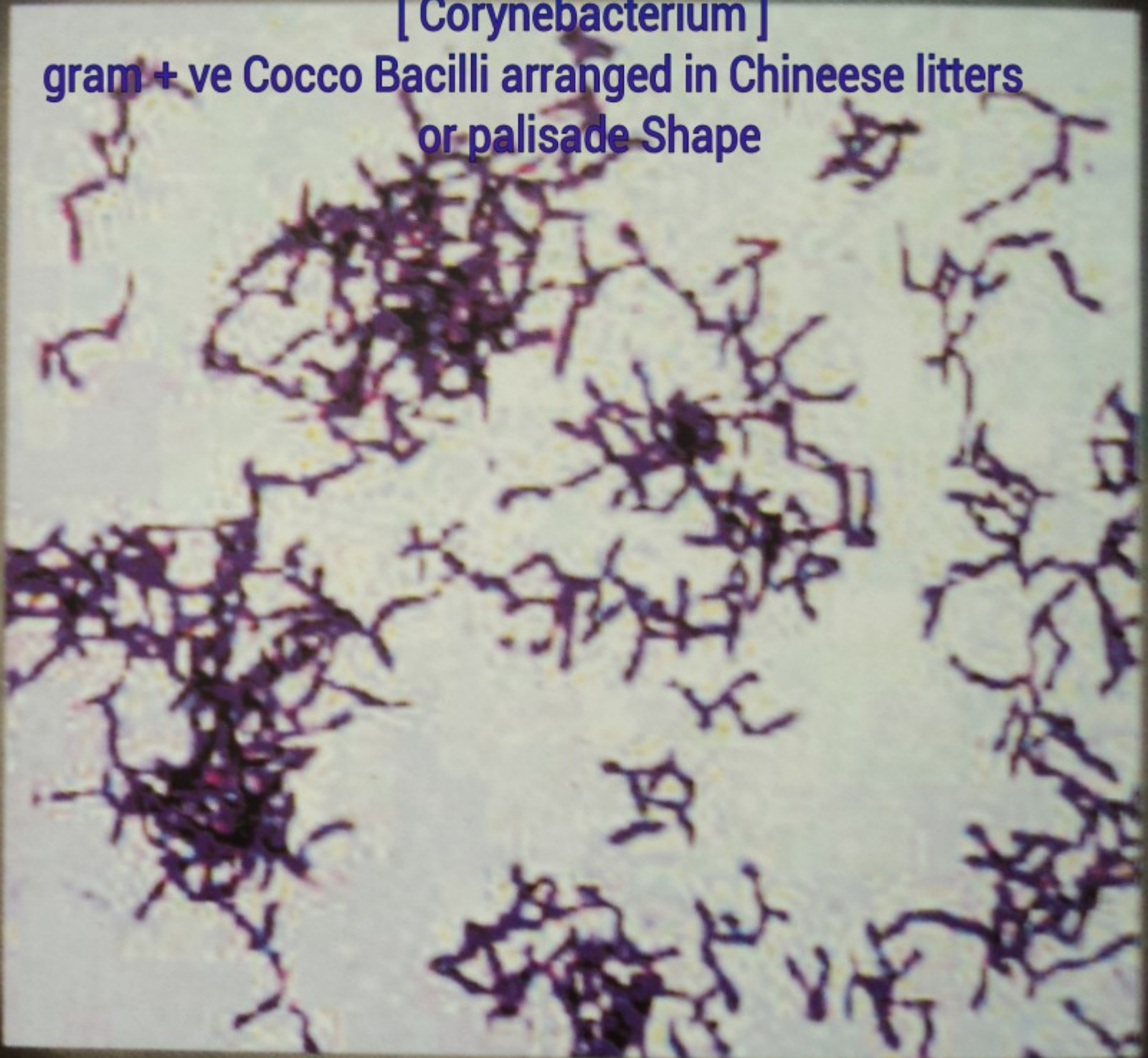
[*Corynebacterium*]

gram + ve Cocco Bacilli arranged in Chinese litters
or palisade Shape



[*Corynebacterium*]

gram + ve Cocco Bacilli arranged in Chinese litters
or palisade Shape



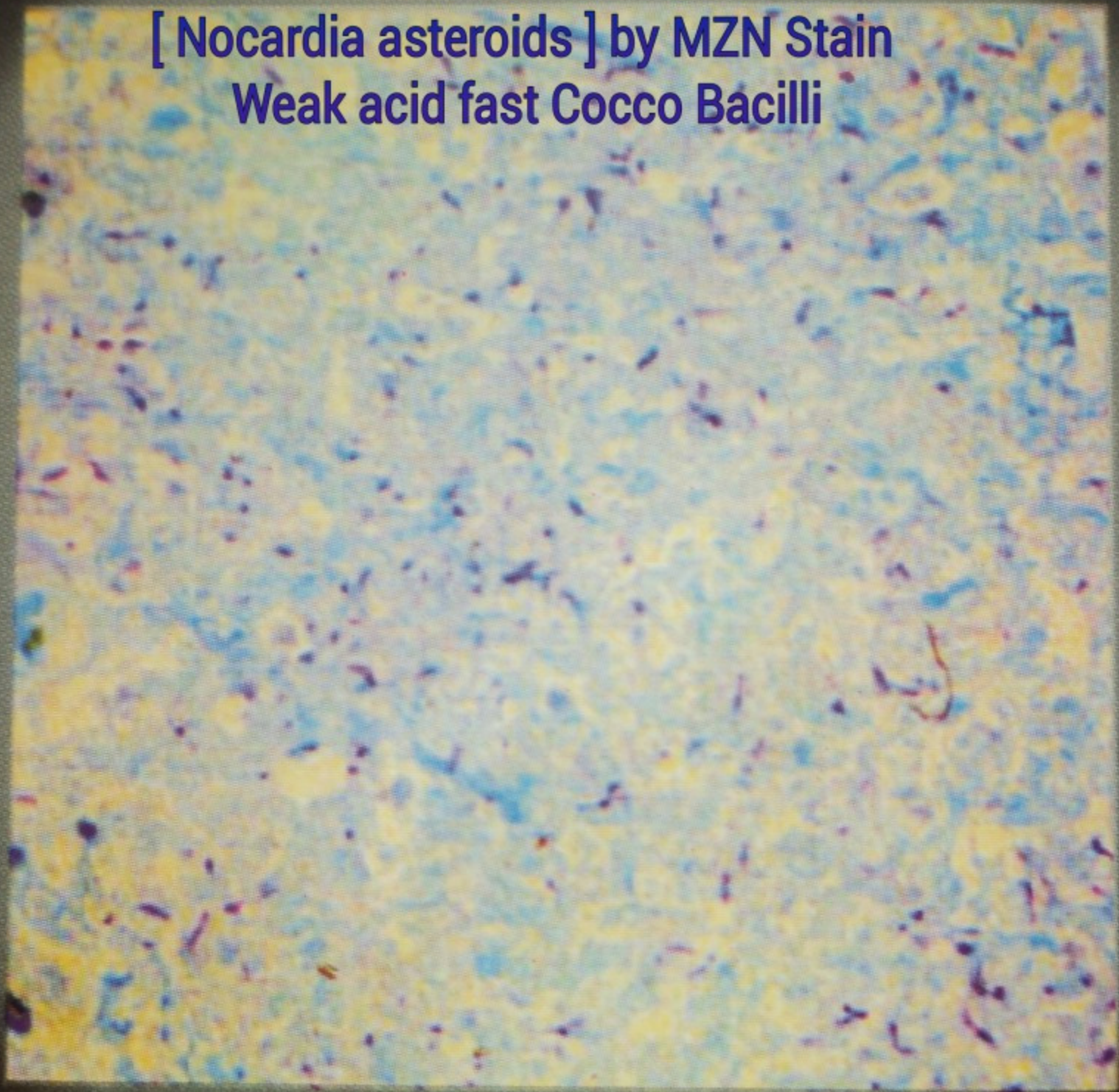
A high-magnification microscopic image showing numerous Listeria bacteria. The bacteria appear as small, pinkish-red, rod-shaped structures (bacilli) scattered across a light-colored background. Some bacteria are arranged in short chains or pairs, while others are isolated. The overall appearance is dense and granular.

[listeria]
gram + ve Cocco Bacilli

[*Rhodococcus equi*]
gram + ve Cocci



[*Nocardia asteroides*] by MZN Stain
Weak acid fast Cocco Bacilli

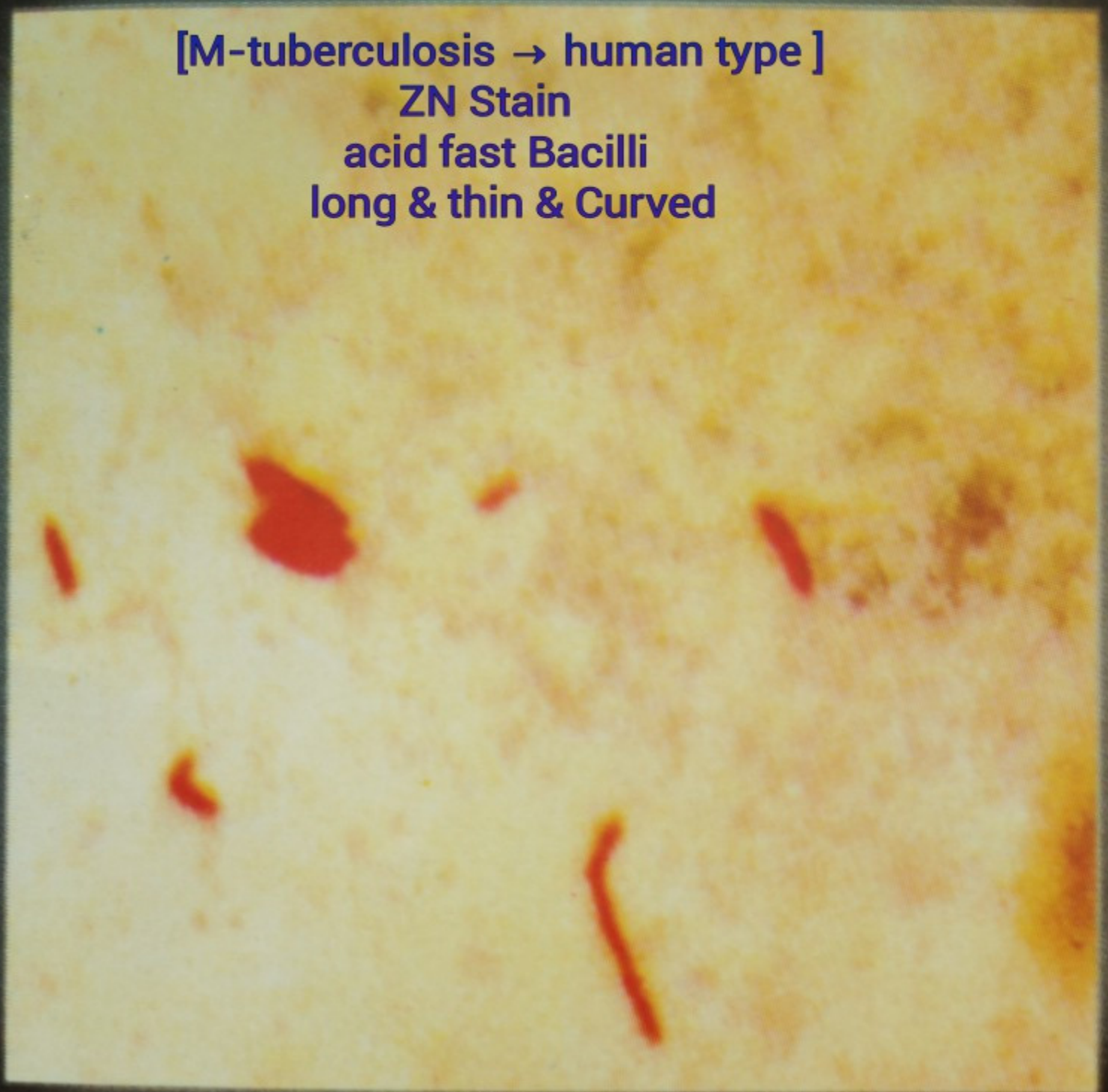


[M-tuberculosis → human type]

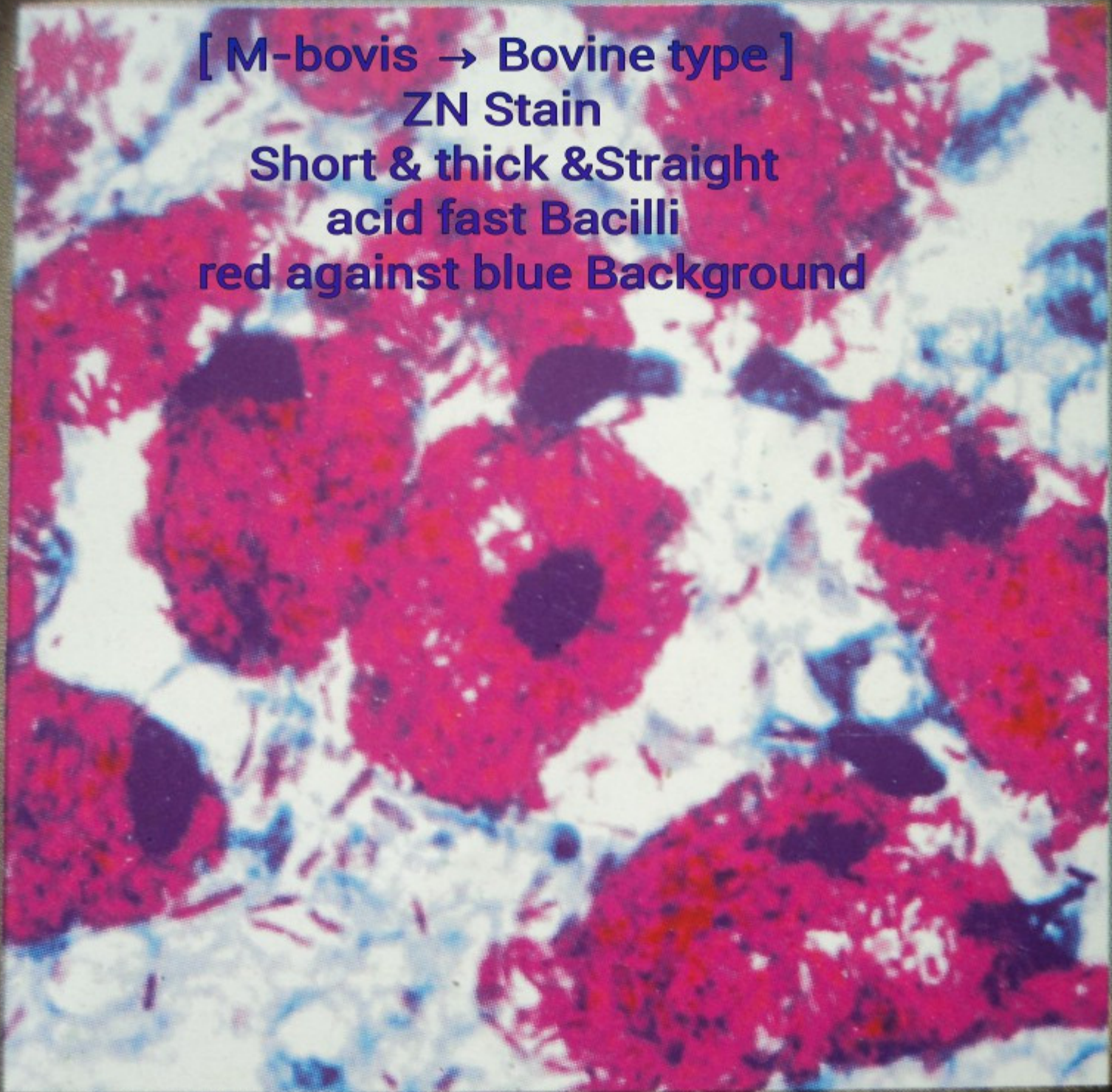
ZN Stain

acid fast Bacilli

long & thin & Curved



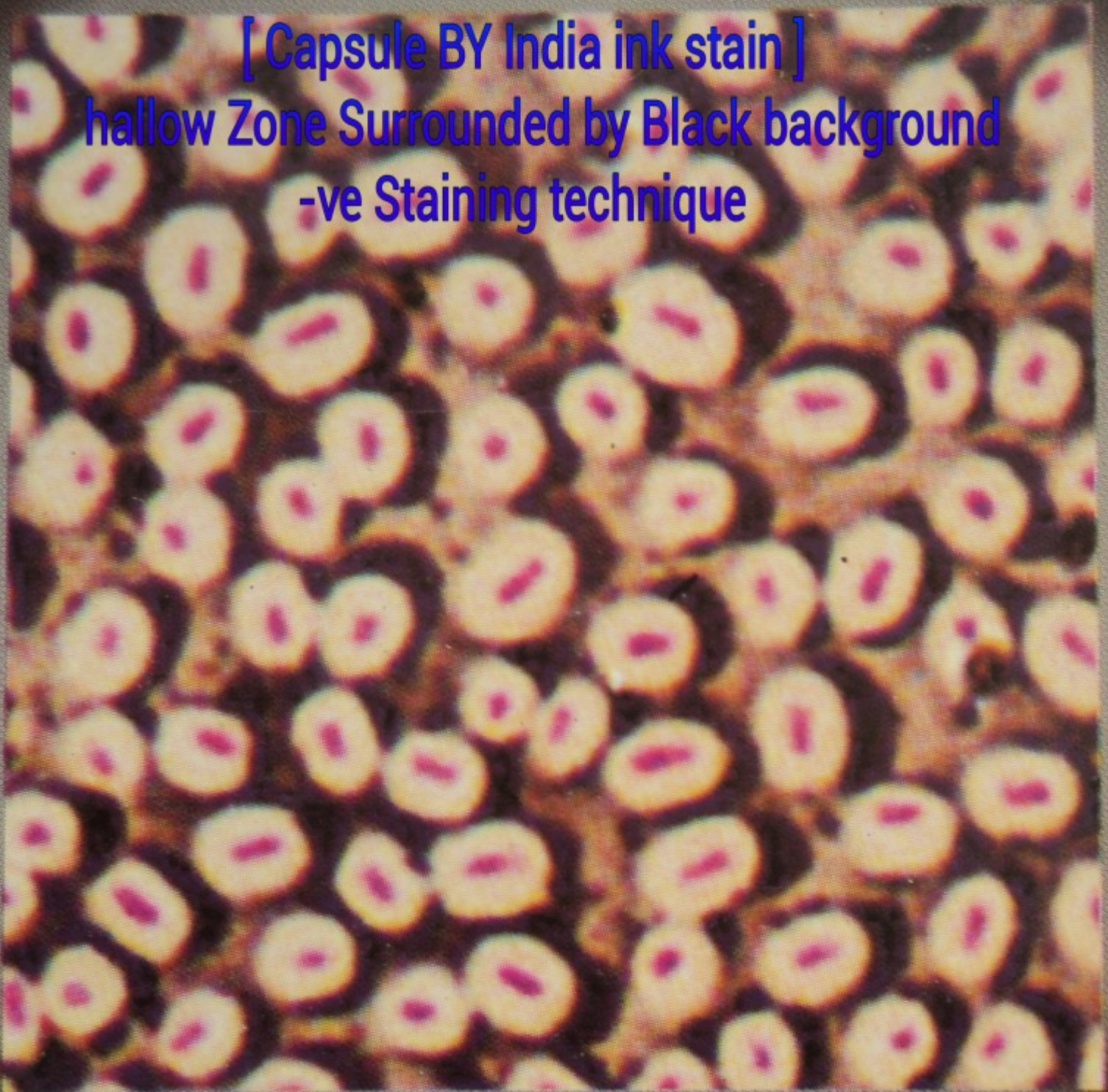
[M-bovis → Bovine type]
ZN Stain
Short & thick & Straight
acid fast Bacilli
red against blue Background

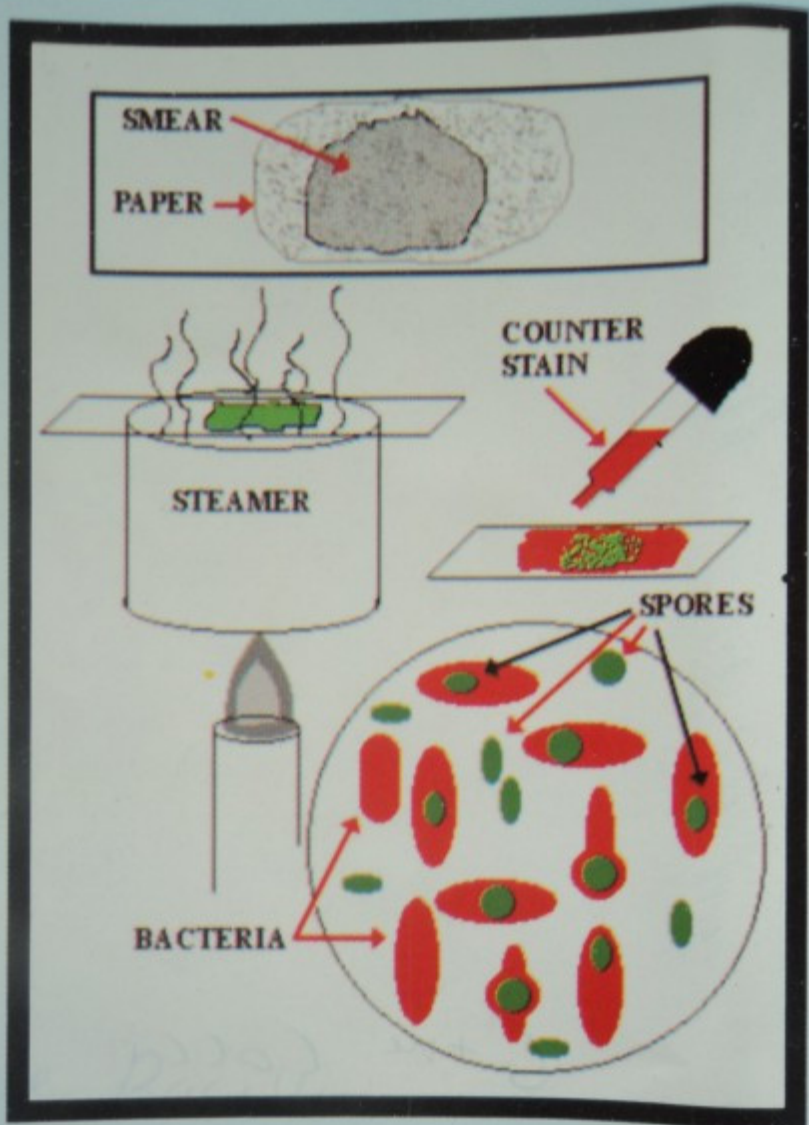


[Capsule BY India ink stain]

hallo Zone Surrounded by Black background

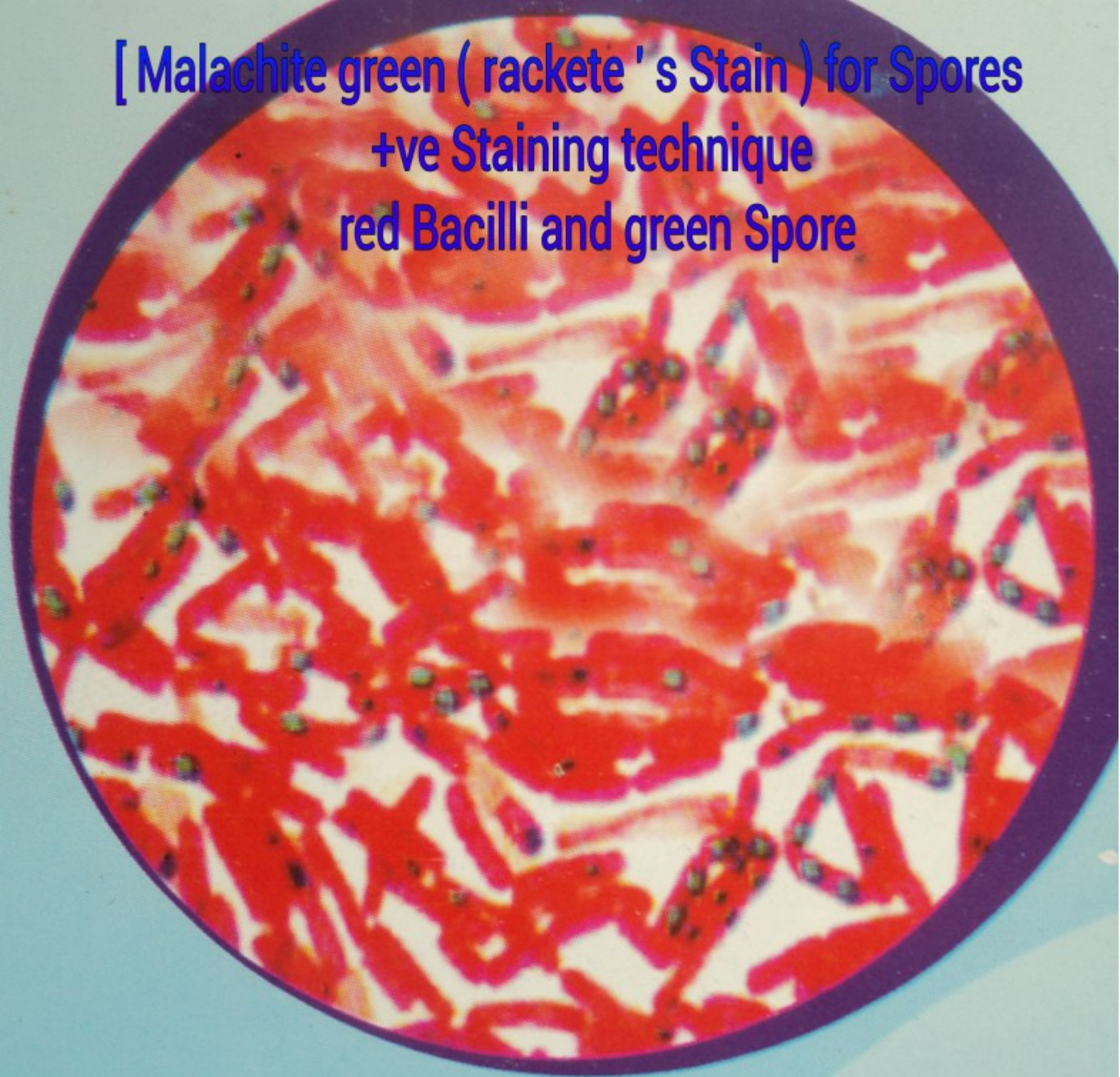
-ve Staining technique





**Steps of staining by
Malachite green
(Rachetes's stain)
for spores**

[Malachite green (rackete ' s Stain) for Spores
+ve Staining technique
red Bacilli and green Spore



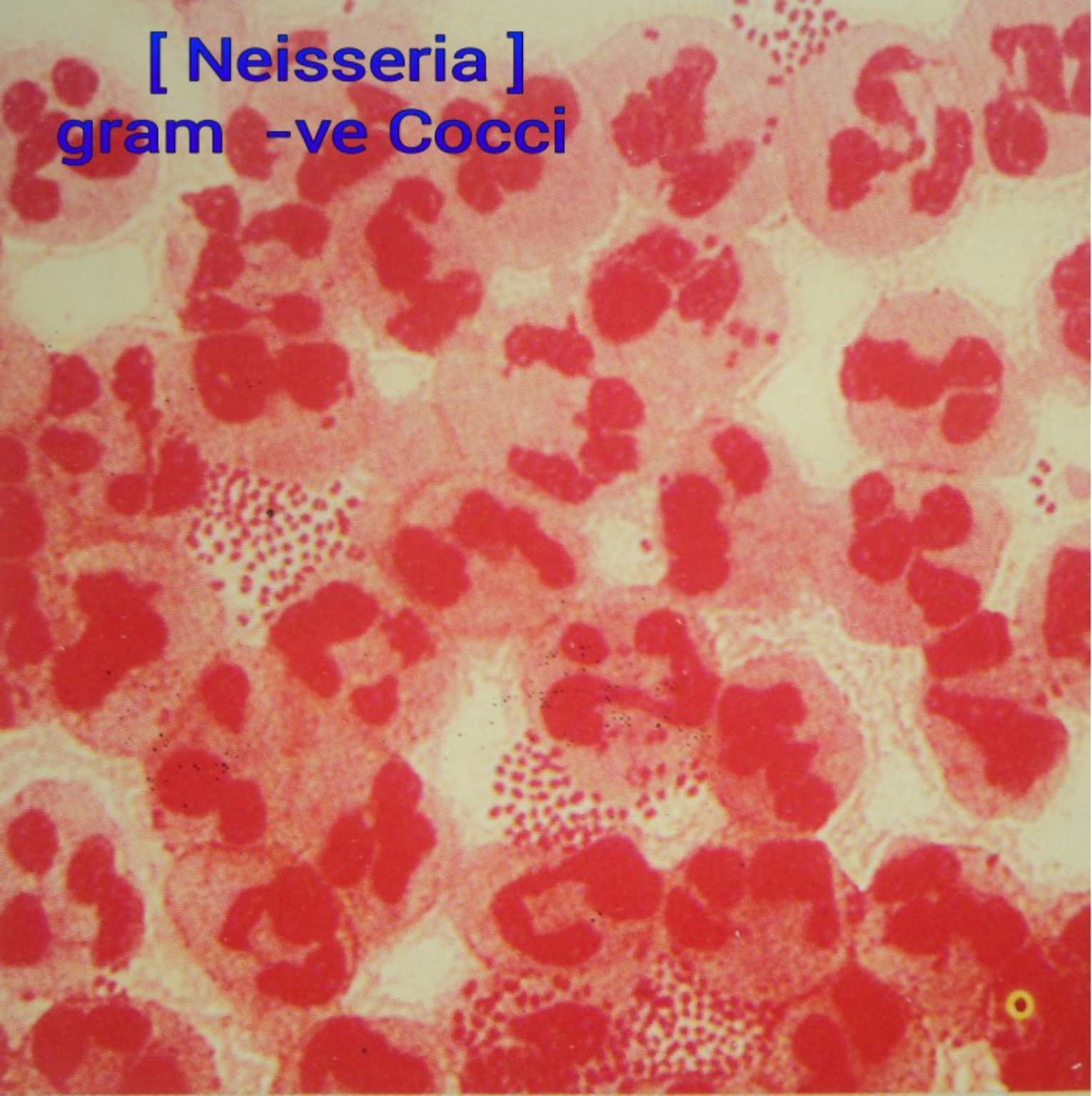
[*Pseudomonas aeruginosa*]
gram -ve Cocco Bacilli



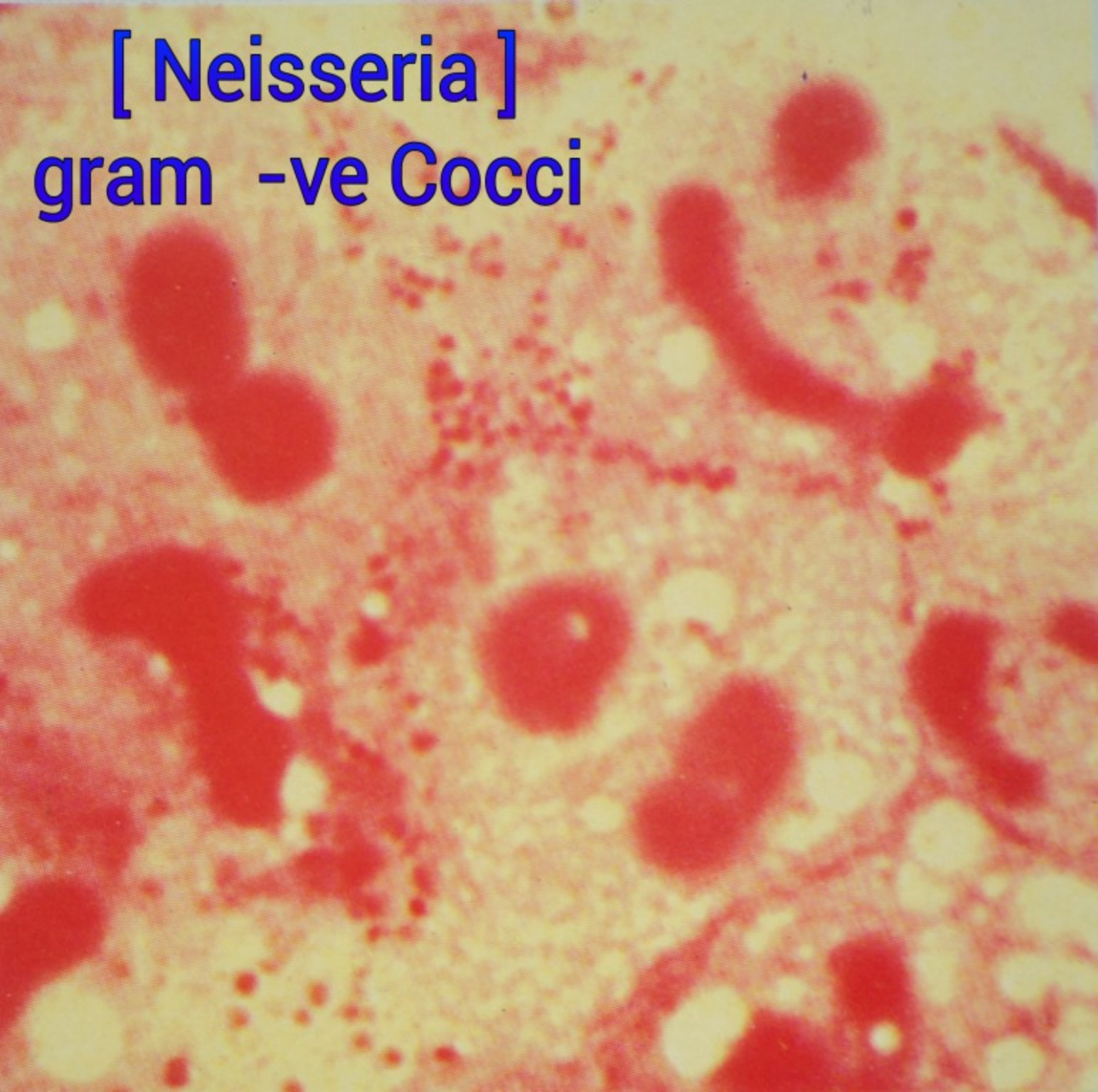
[*Moraxella bovis*]
gram -ve Thick bacilli



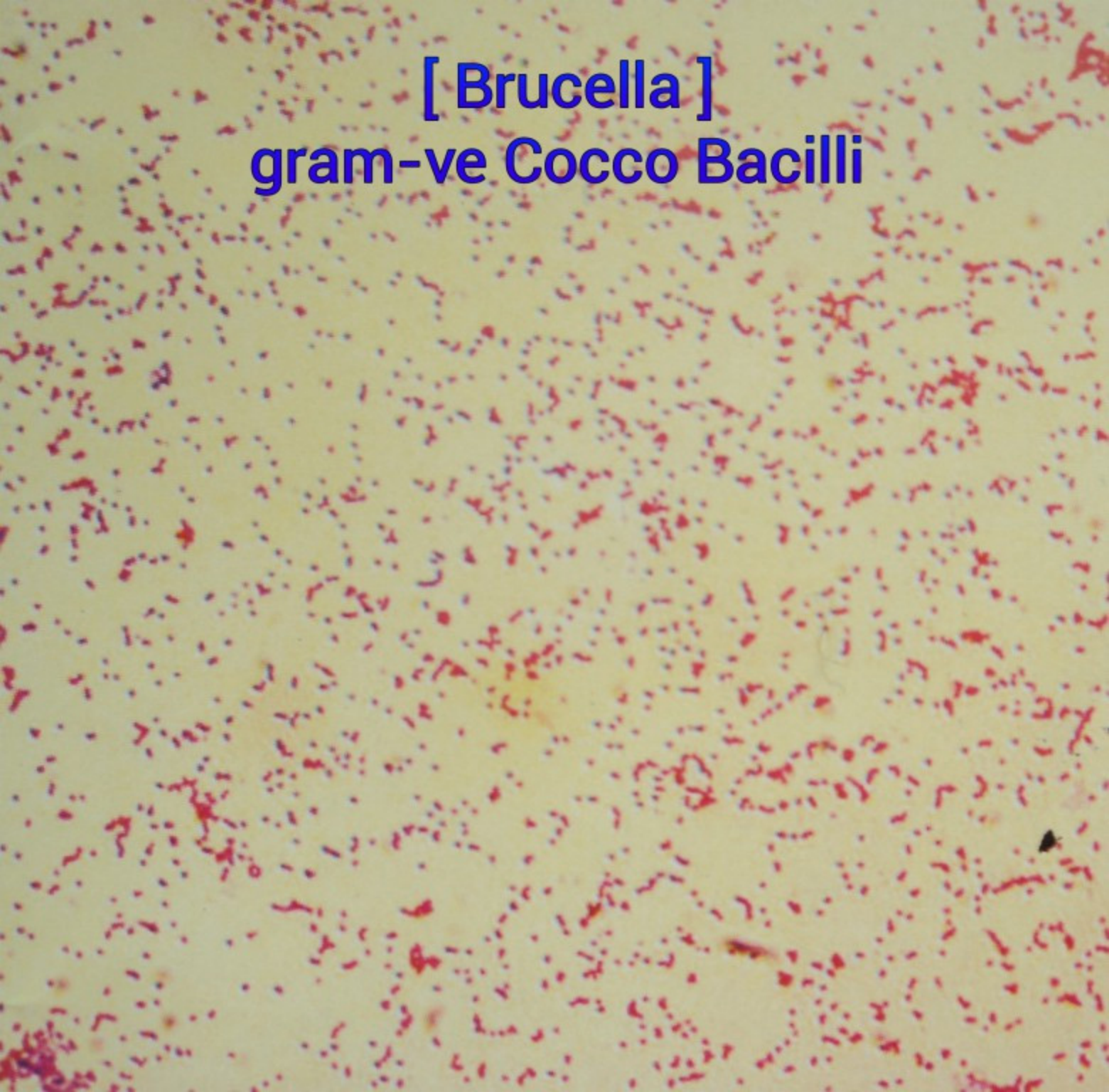
[Neisseria]
gram -ve Cocci



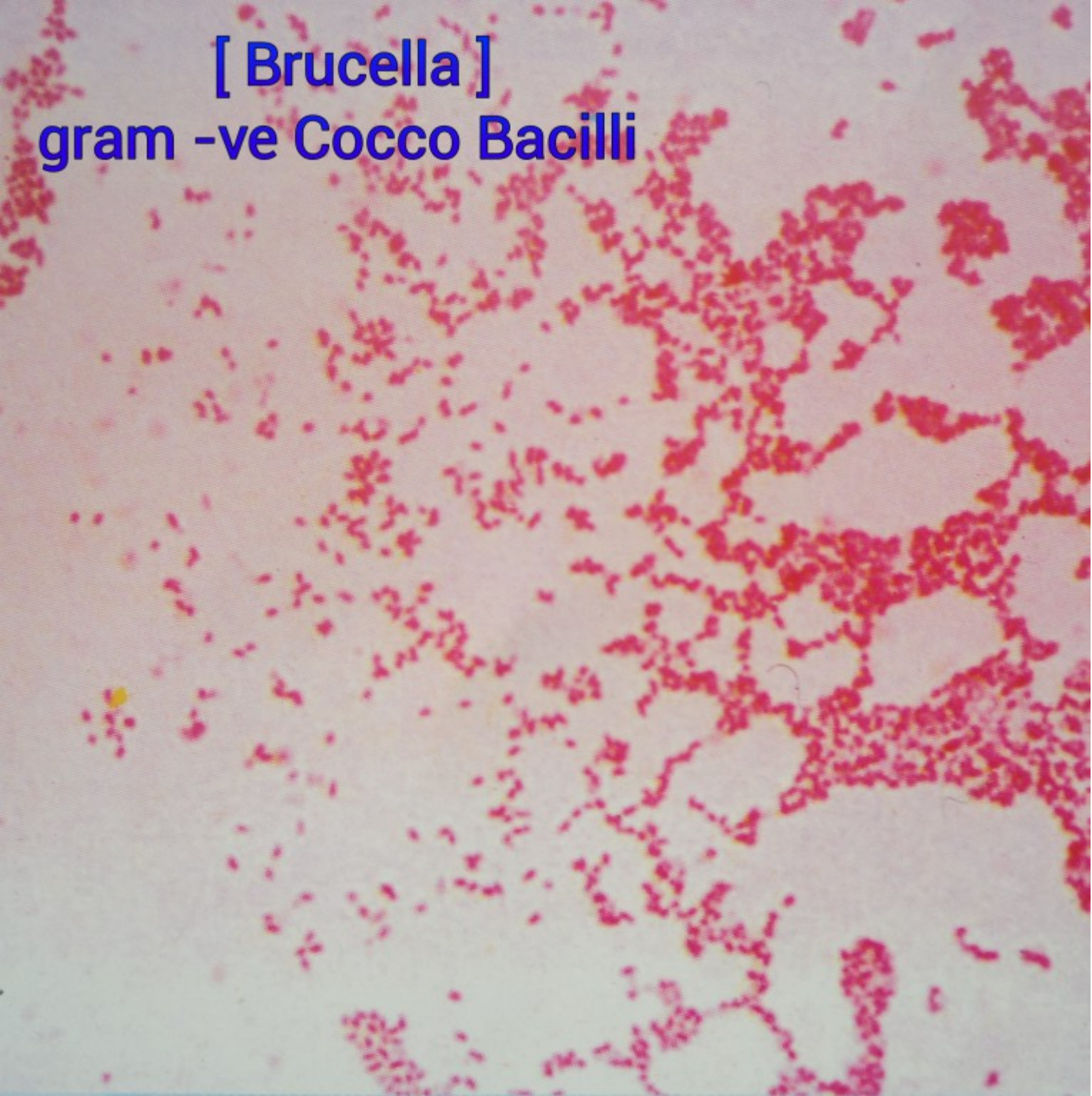
[Neisseria]
gram -ve Cocci



[Brucella]
gram-ve Cocco Bacilli

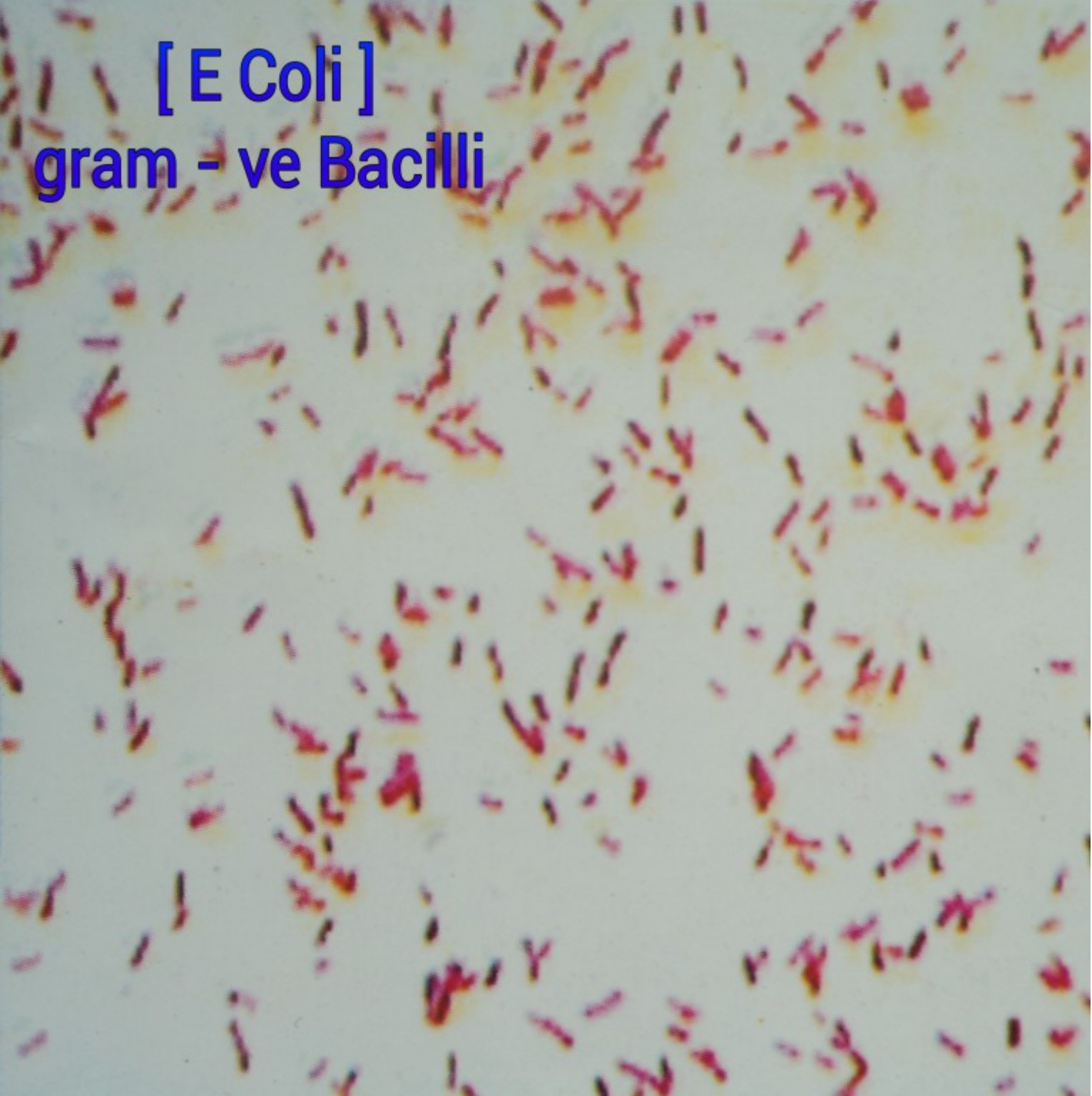


[Brucella]
gram -ve Cocco Bacilli

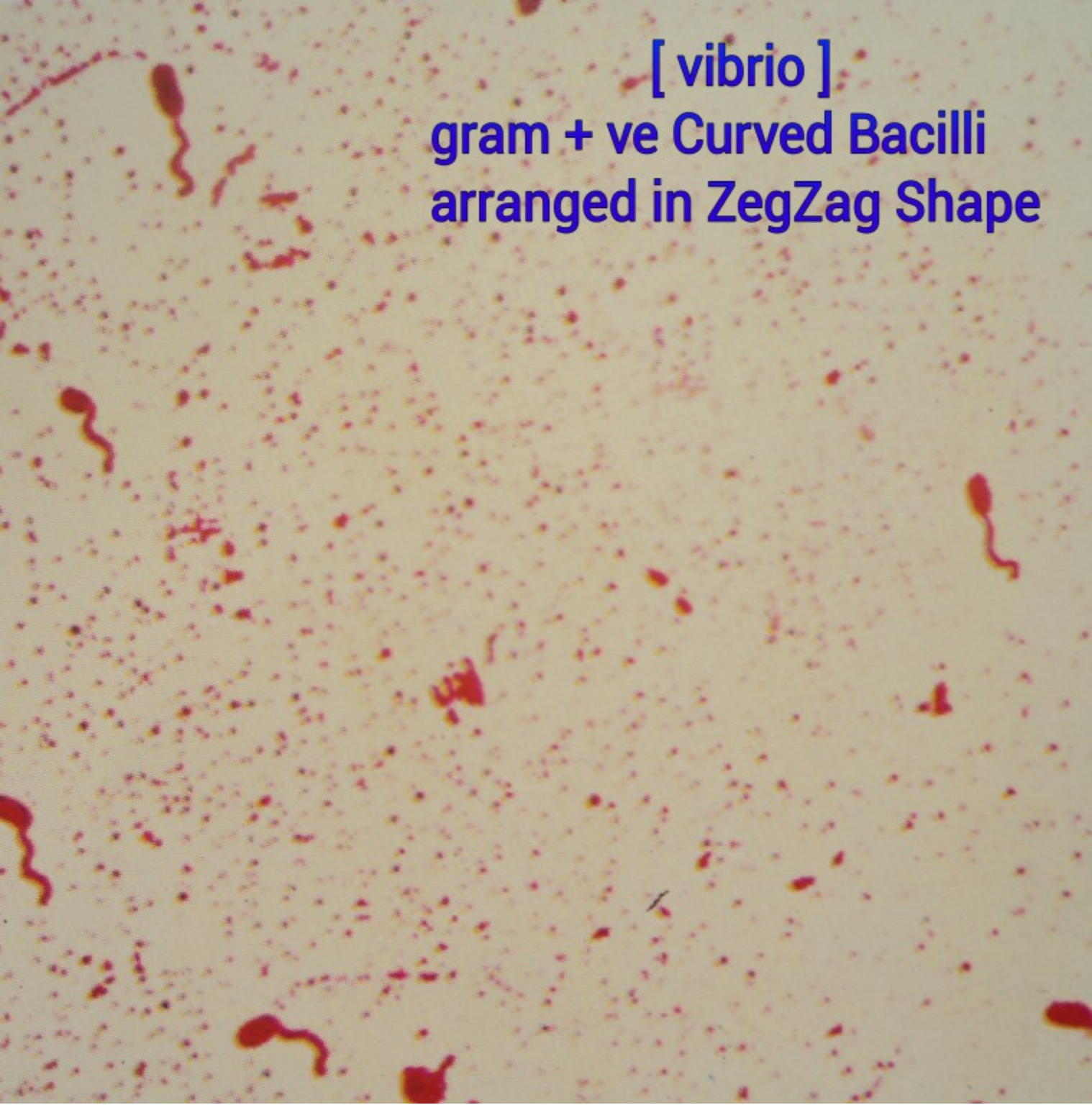


[E Coli]

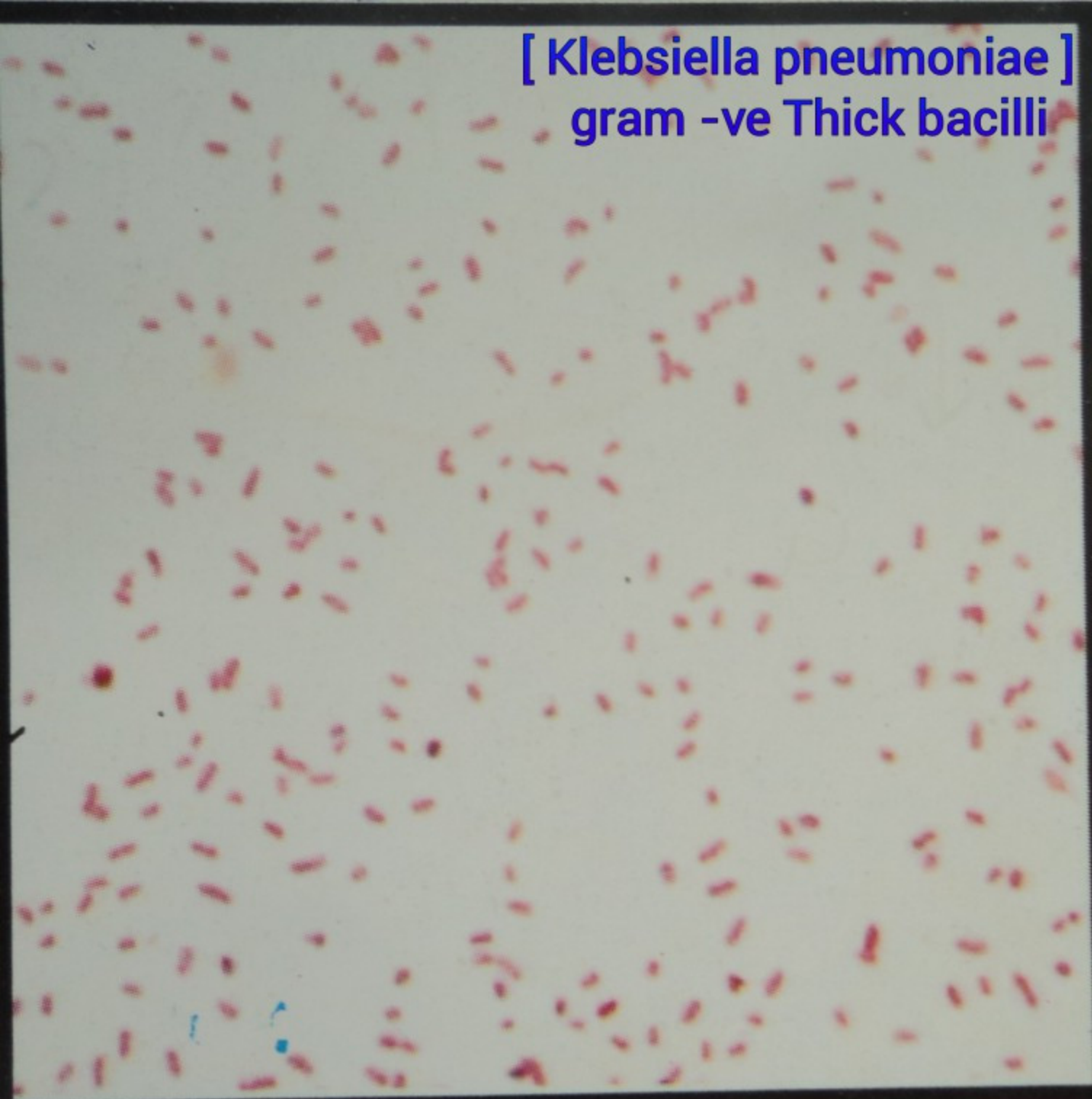
gram - ve Bacilli



[vibrio]
gram + ve Curved Bacilli
arranged in ZegZag Shape

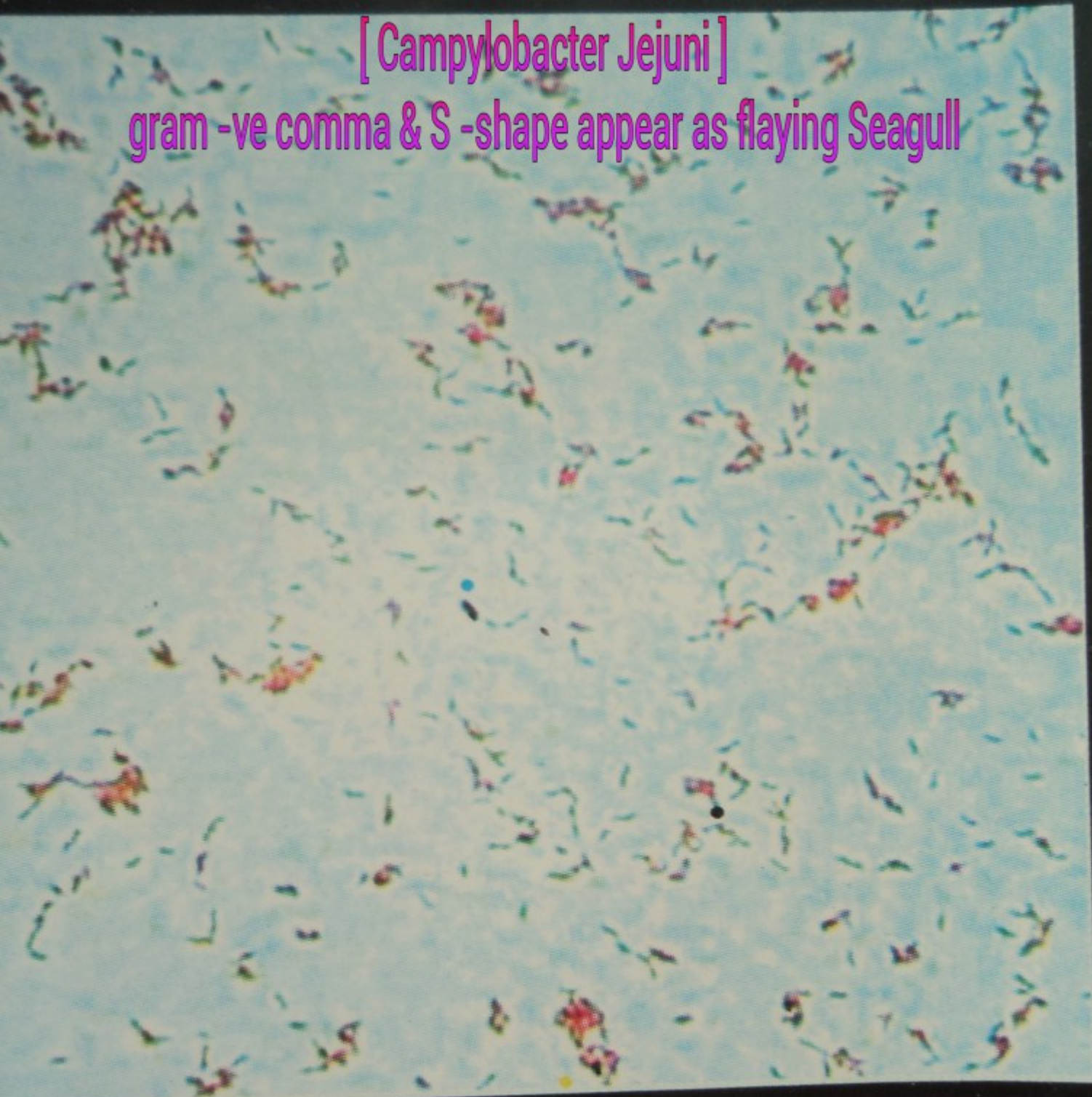


[*Klebsiella pneumoniae*]
gram -ve Thick bacilli

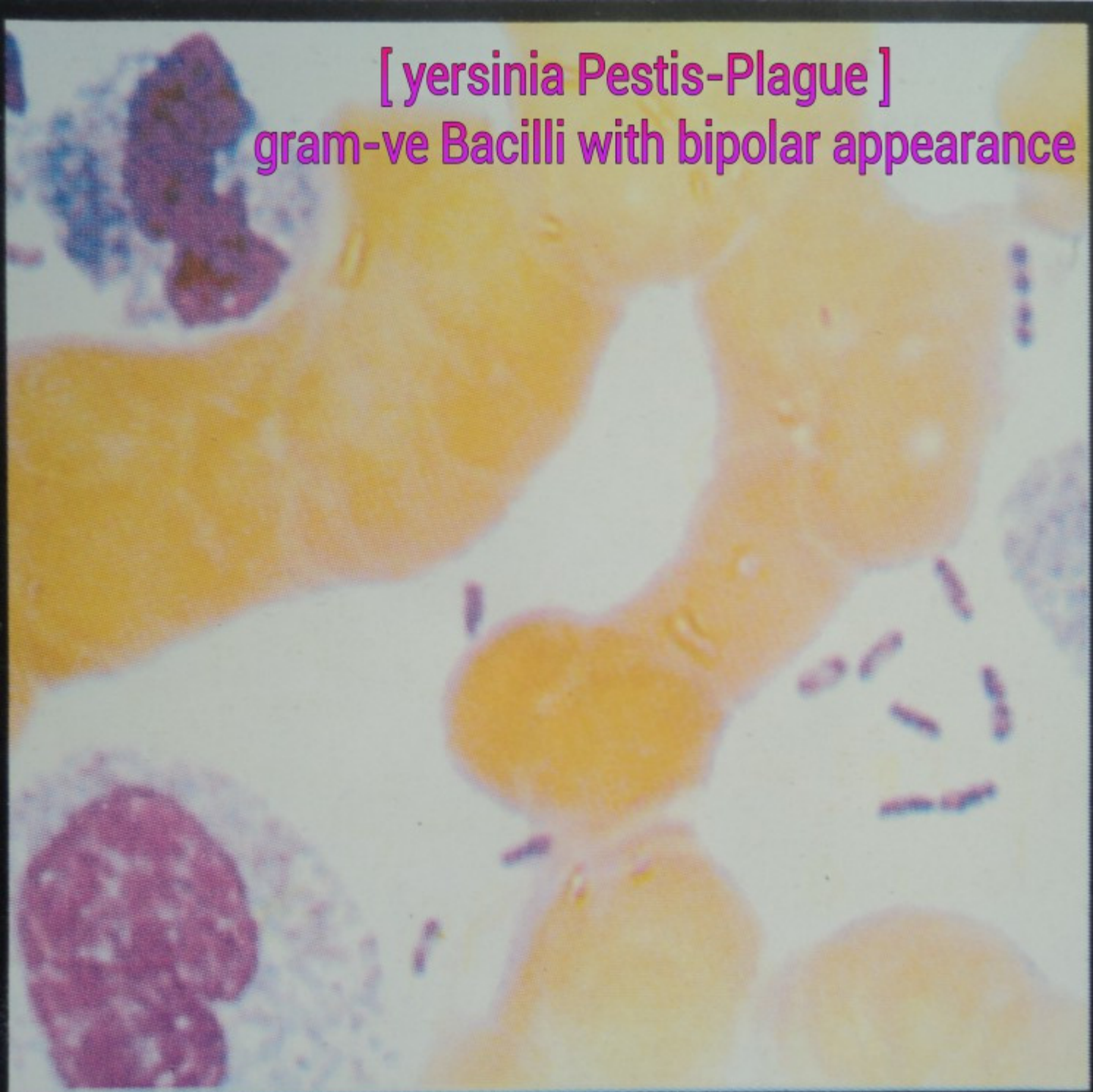


[*Campylobacter Jejuni*]

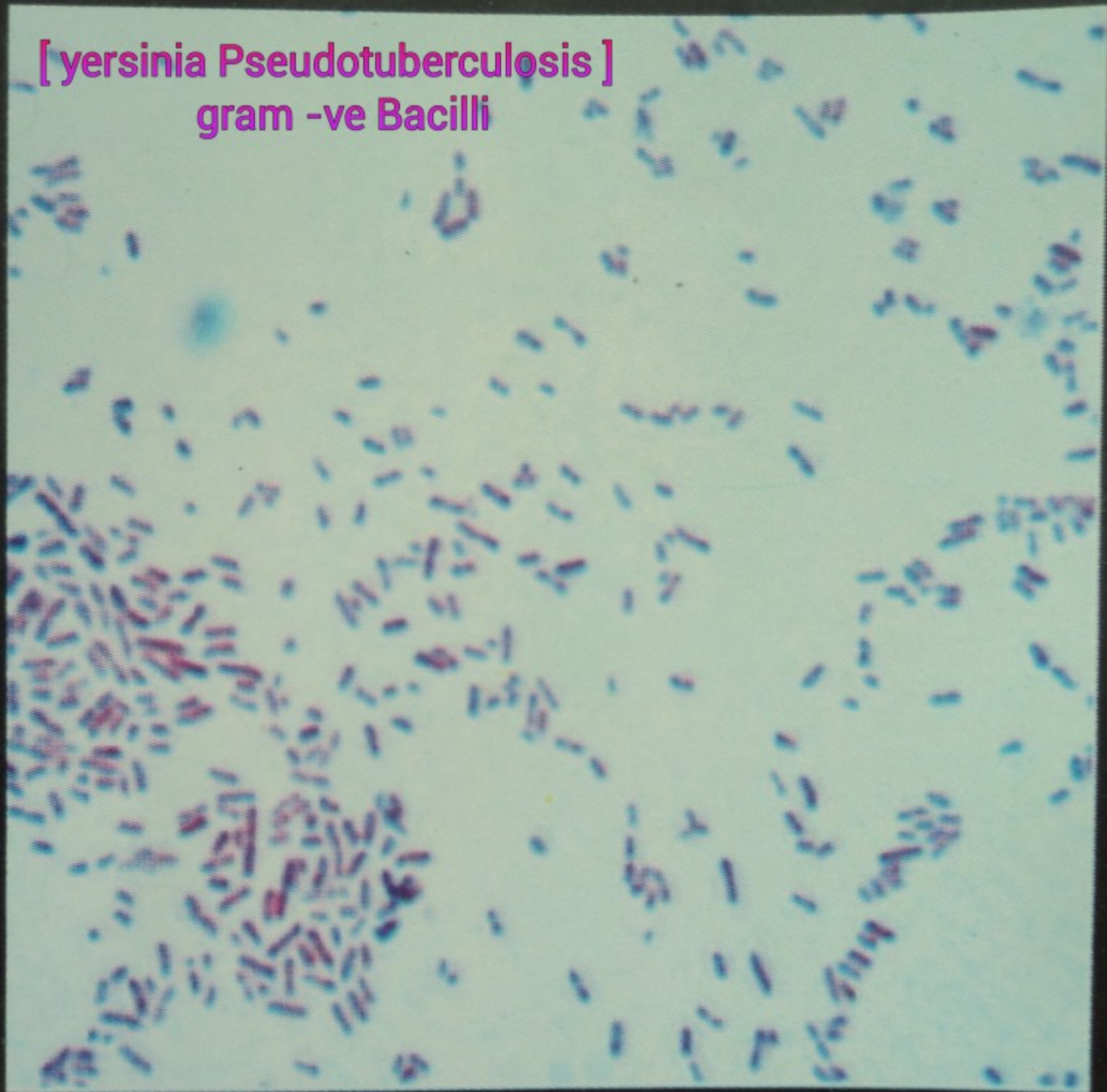
gram -ve comma & S -shape appear as flying Seagull



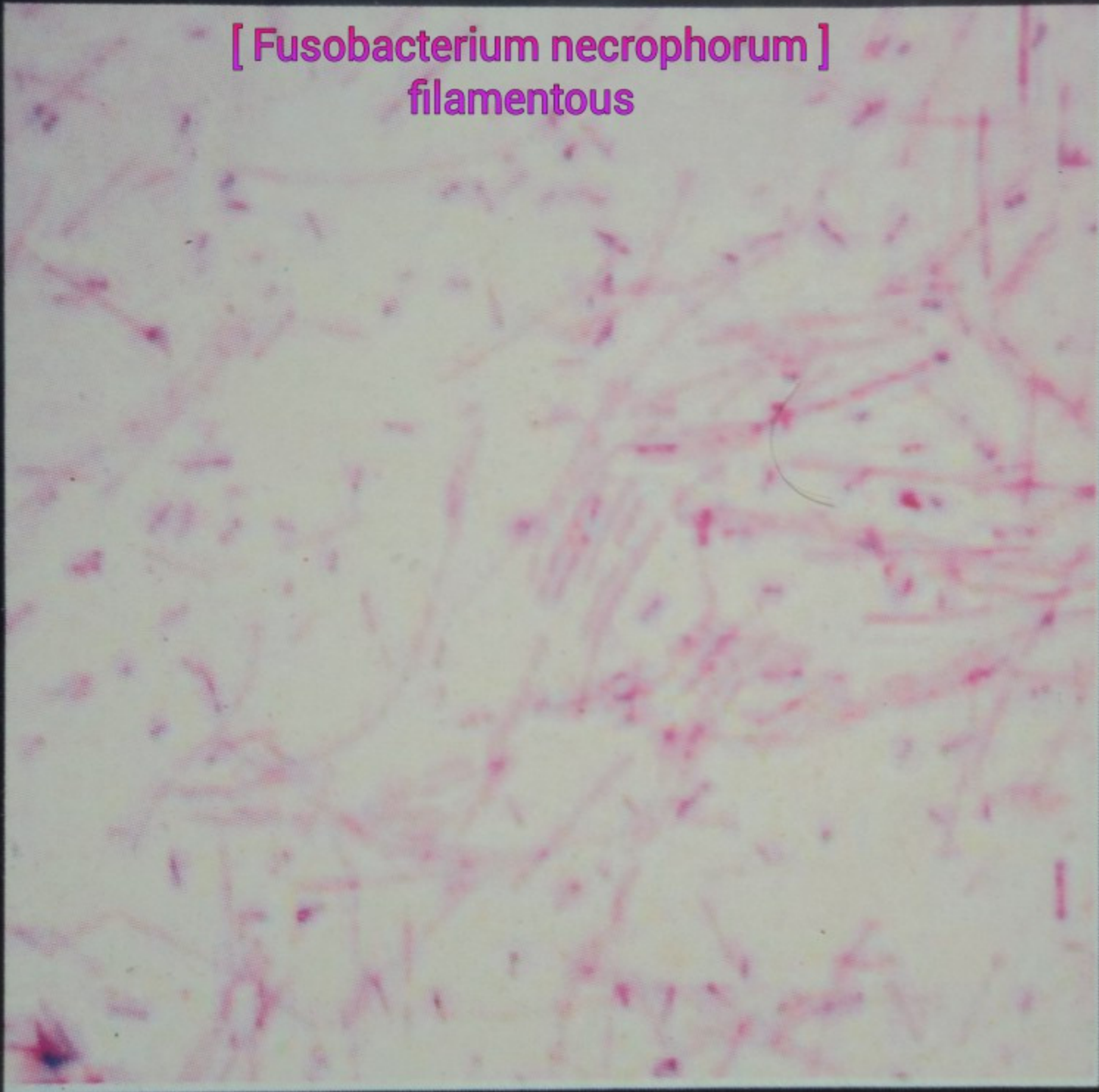
[yersinia Pestis-Plague]
gram-ve Bacilli with bipolar appearance



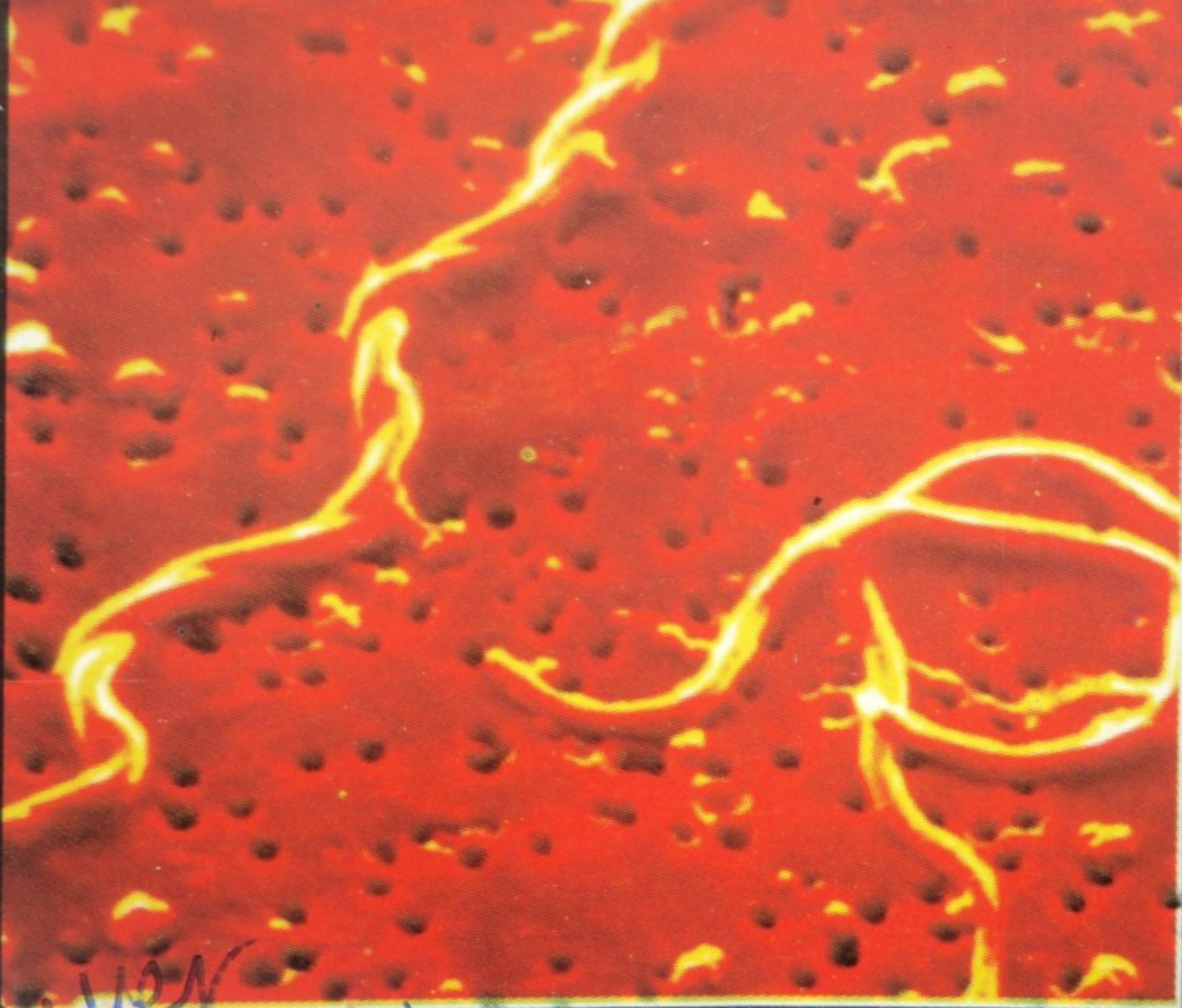
[yersinia Pseudotuberculosis]
gram -ve Bacilli



[*Fusobacterium necrophorum*]
filamentous

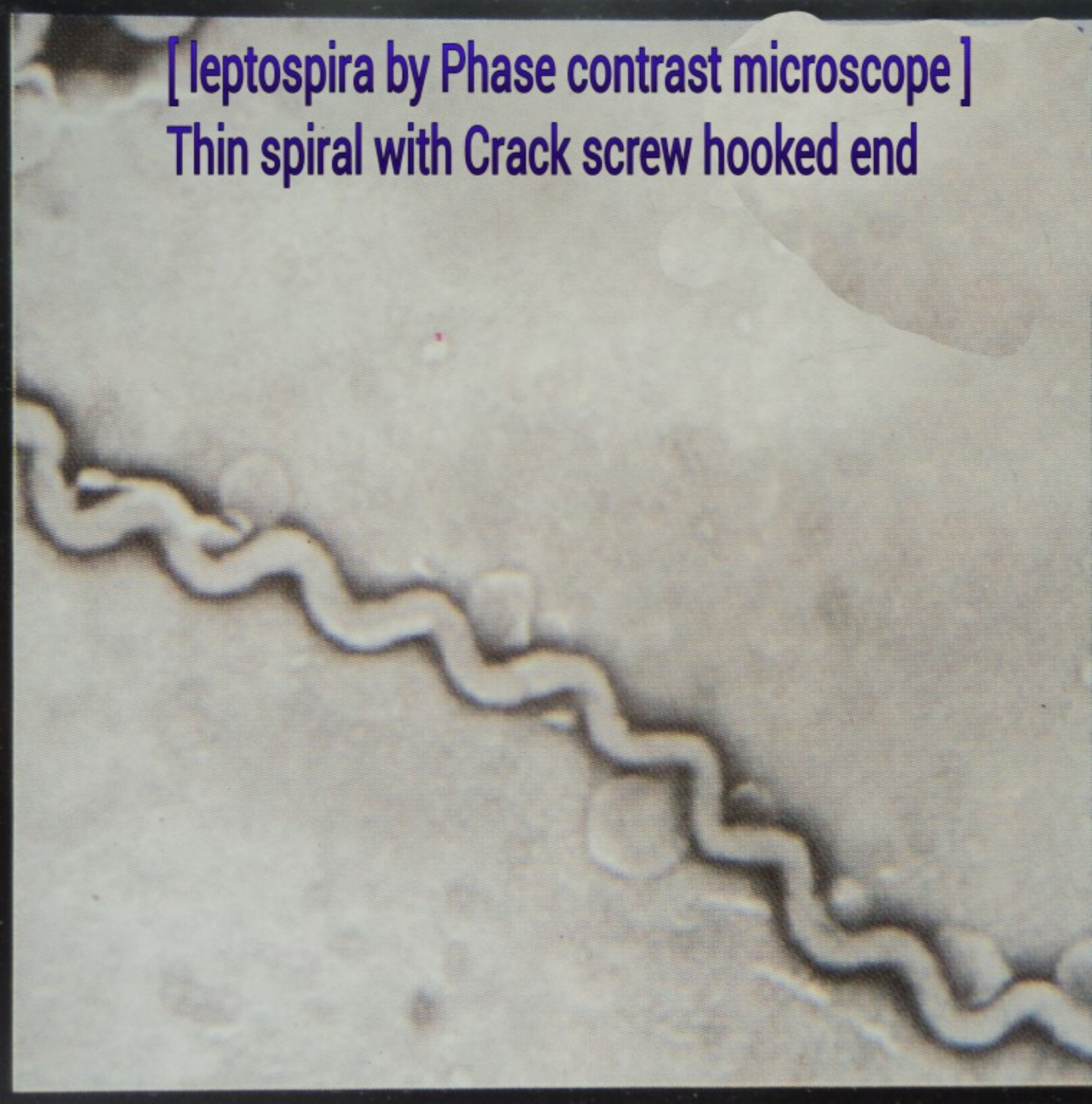


[leptospira by Silver impregnation Stain]
Thin spiral with Crack screw hooked end



WON

[leptospira by Phase contrast microscope]
Thin spiral with Crack screw hooked end



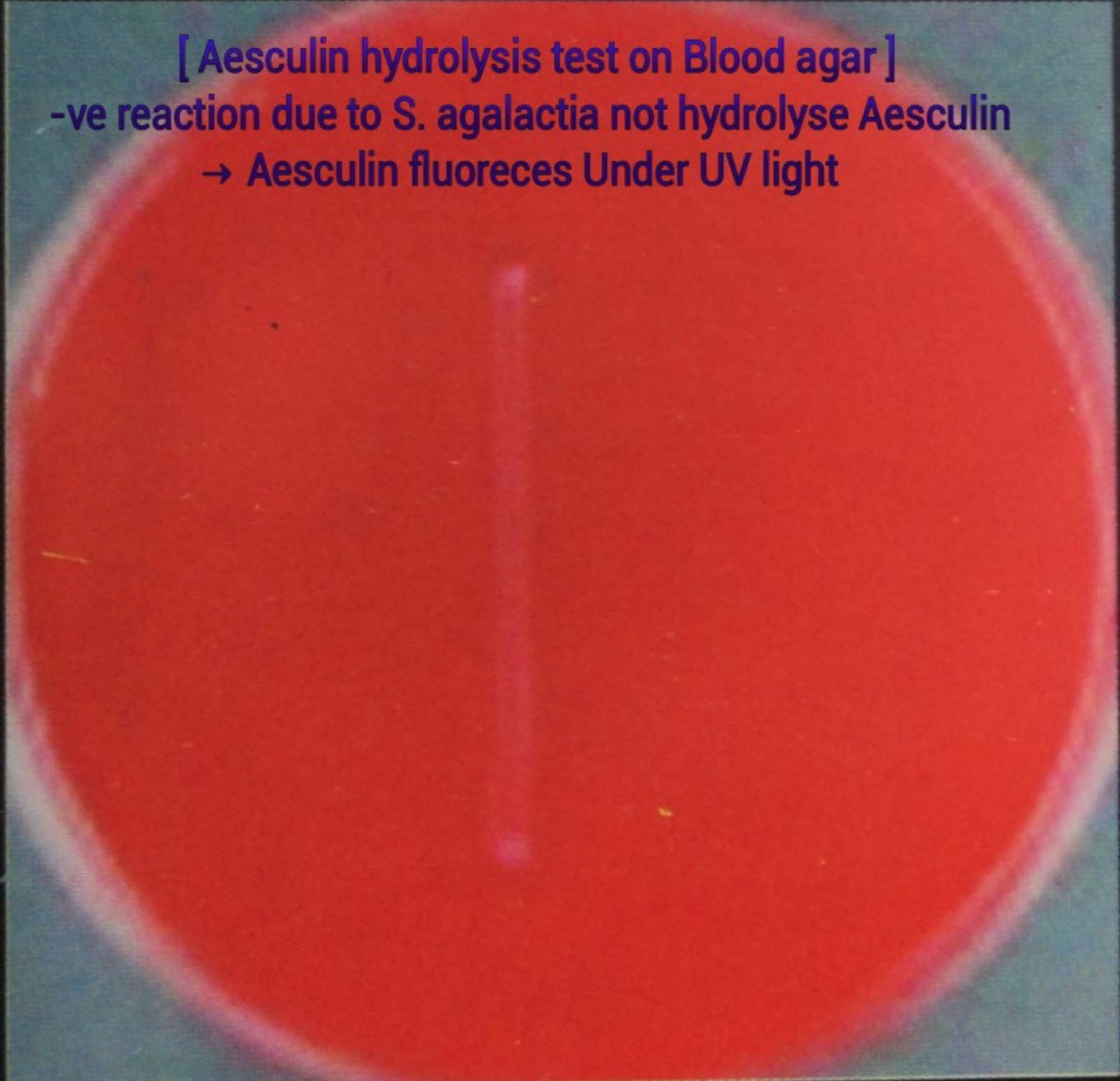


[Borrelia]

Thick spiral bacteria

[Aesculin hydrolysis test on Blood agar]

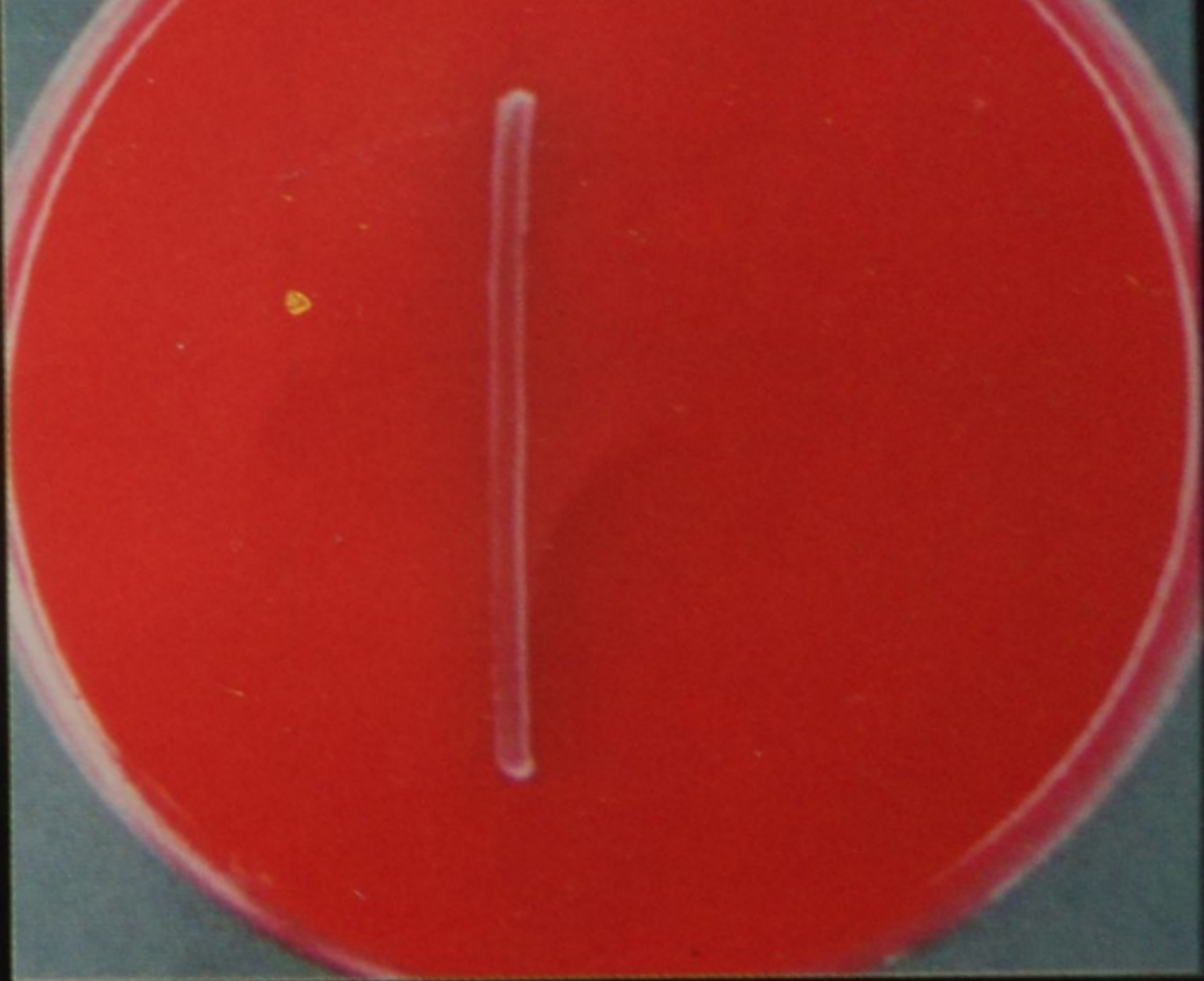
**-ve reaction due to *S. agalactia* not hydrolyse Aesculin
→ Aesculin fluoresces Under UV light**



[Aesculin hydrolysis test on Blood agar]

+Ve reaction due to *E. faecalis* hydrolyse Aesculin

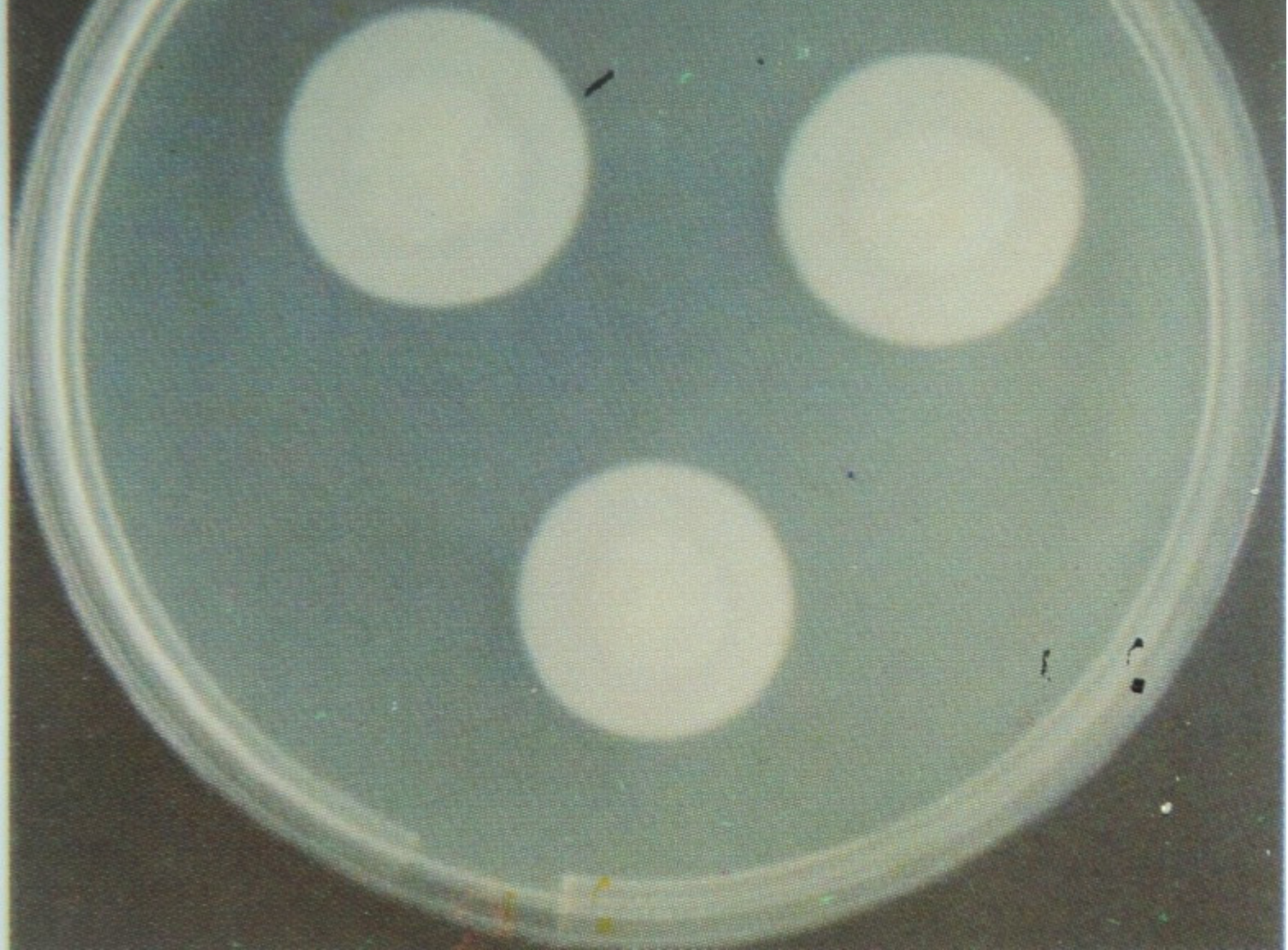
→ Aesculin not fluoresces under UV light



[lecithinase test]

Media Containing egg Yolk

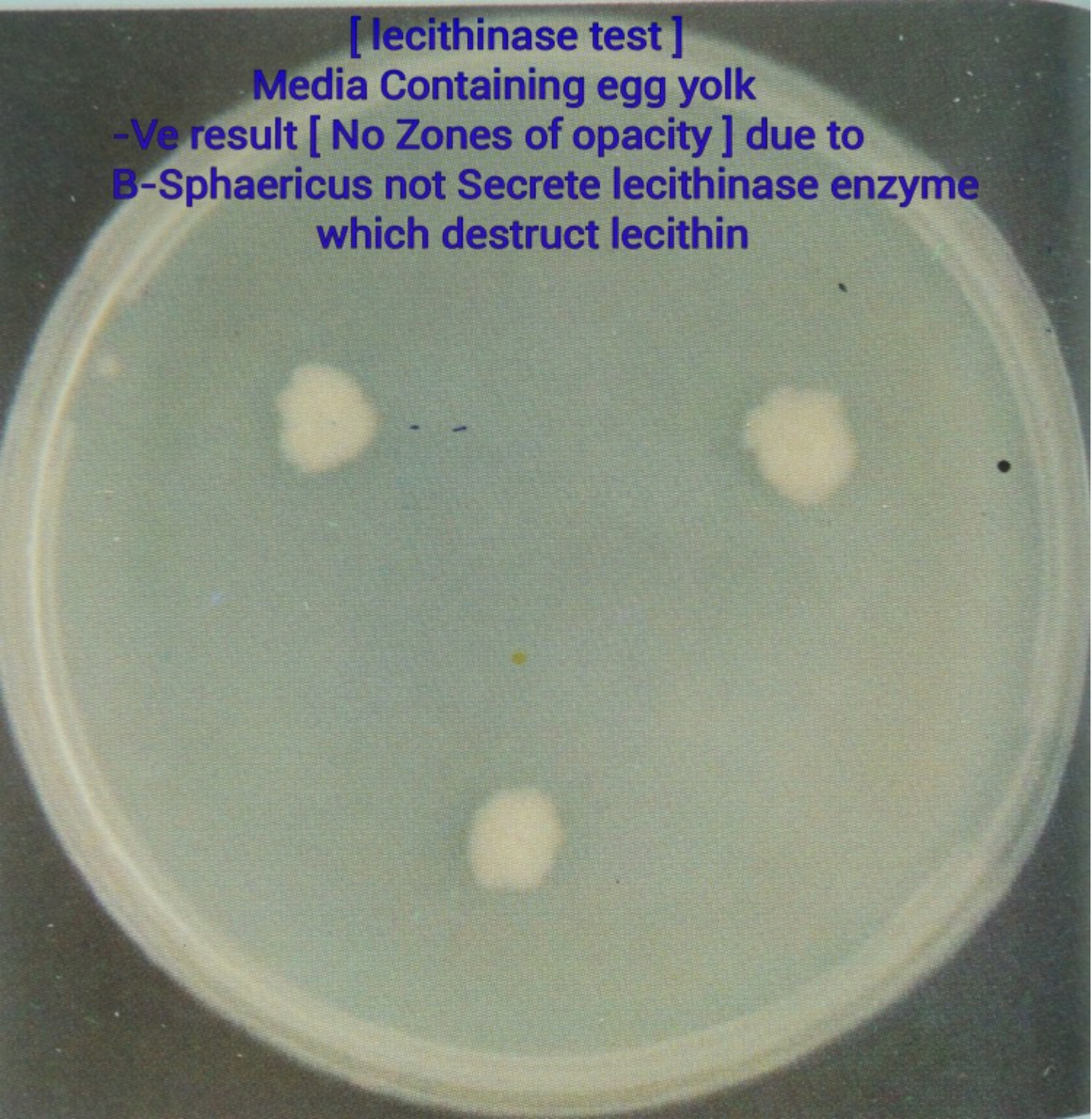
**+ ve result [Zones of opacity] by B-Cereus
which Secretes lecithinase enzyme which
destruct protein**



[lecithinase test]

Media Containing egg yolk

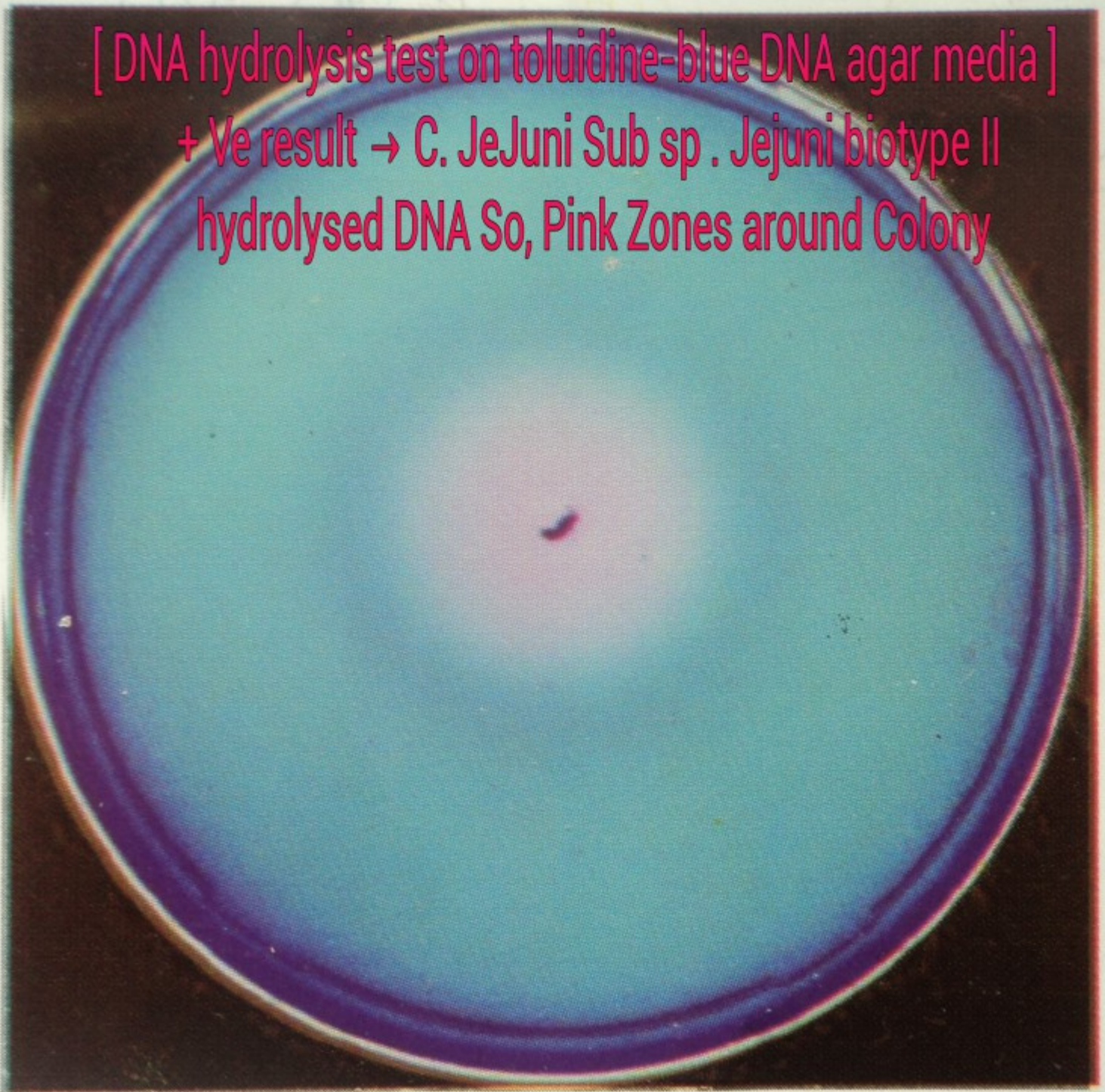
**-Ve result [No Zones of opacity] due to
B-Sphaericus not Secrete lecithinase enzyme
which destruct lecithin**



[DNA hydrolysis test on toluidine - blue DNA agar media]
-ve result → One of the thermophilic Campylobacters not
hydrolyse DNA So, No Pink Zone around Colony



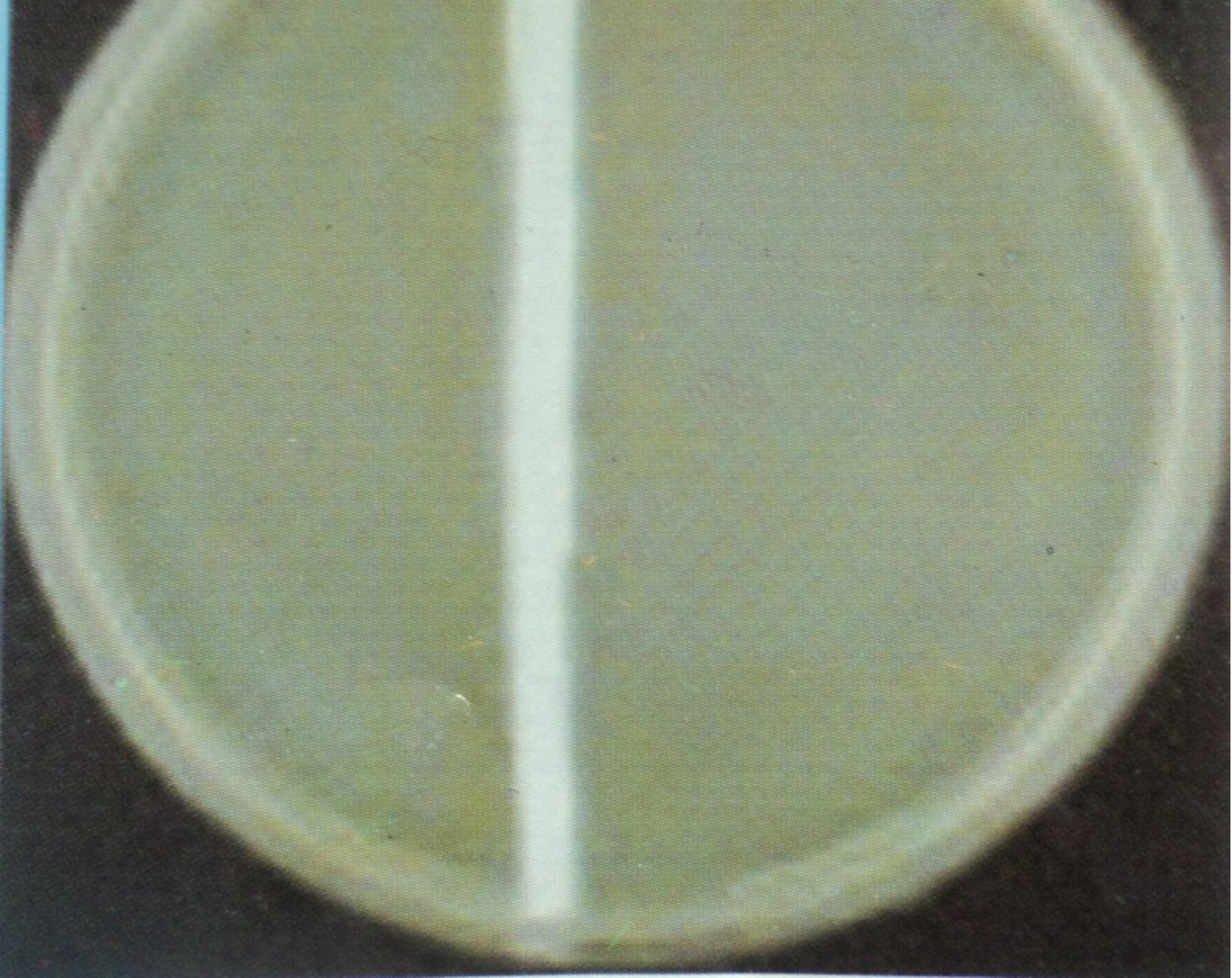
[DNA hydrolysis test on toluidine-blue DNA agar media]
+ Ve result → C. JeJuni Sub sp . Jejuni biotype II
hydrolysed DNA So, Pink Zones around Colony



[gelatin hydrolysis test]

indicator → Sulpho salicylic acid

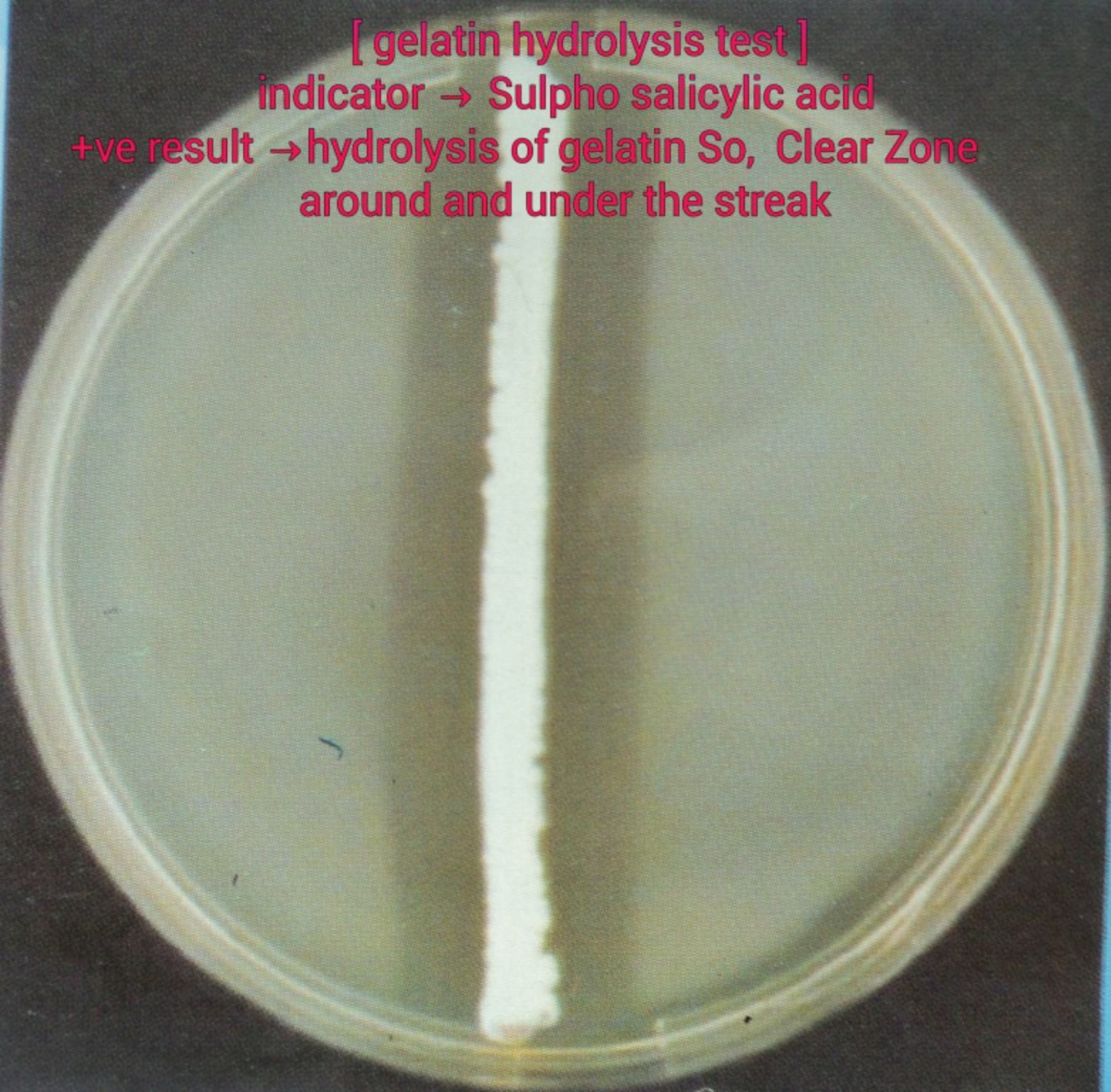
-ve result → no hydrolysis of gelatin So, no Clear Zone
around and under the streak



[gelatin hydrolysis test]

indicator → Sulpho salicylic acid

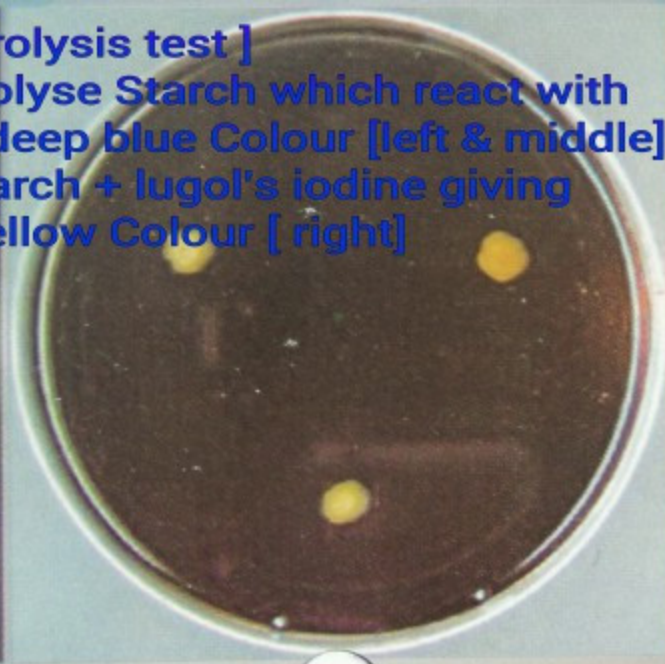
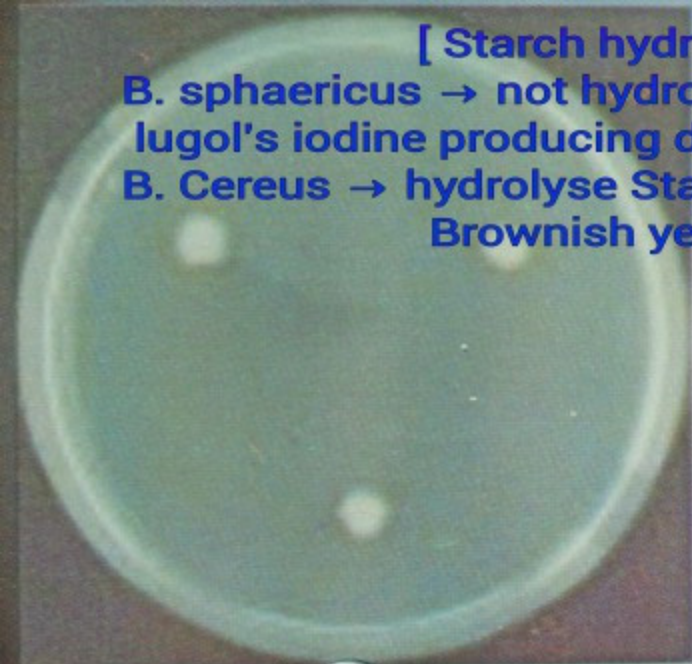
+ve result → hydrolysis of gelatin So, Clear Zone
around and under the streak



[Starch hydrolysis test]

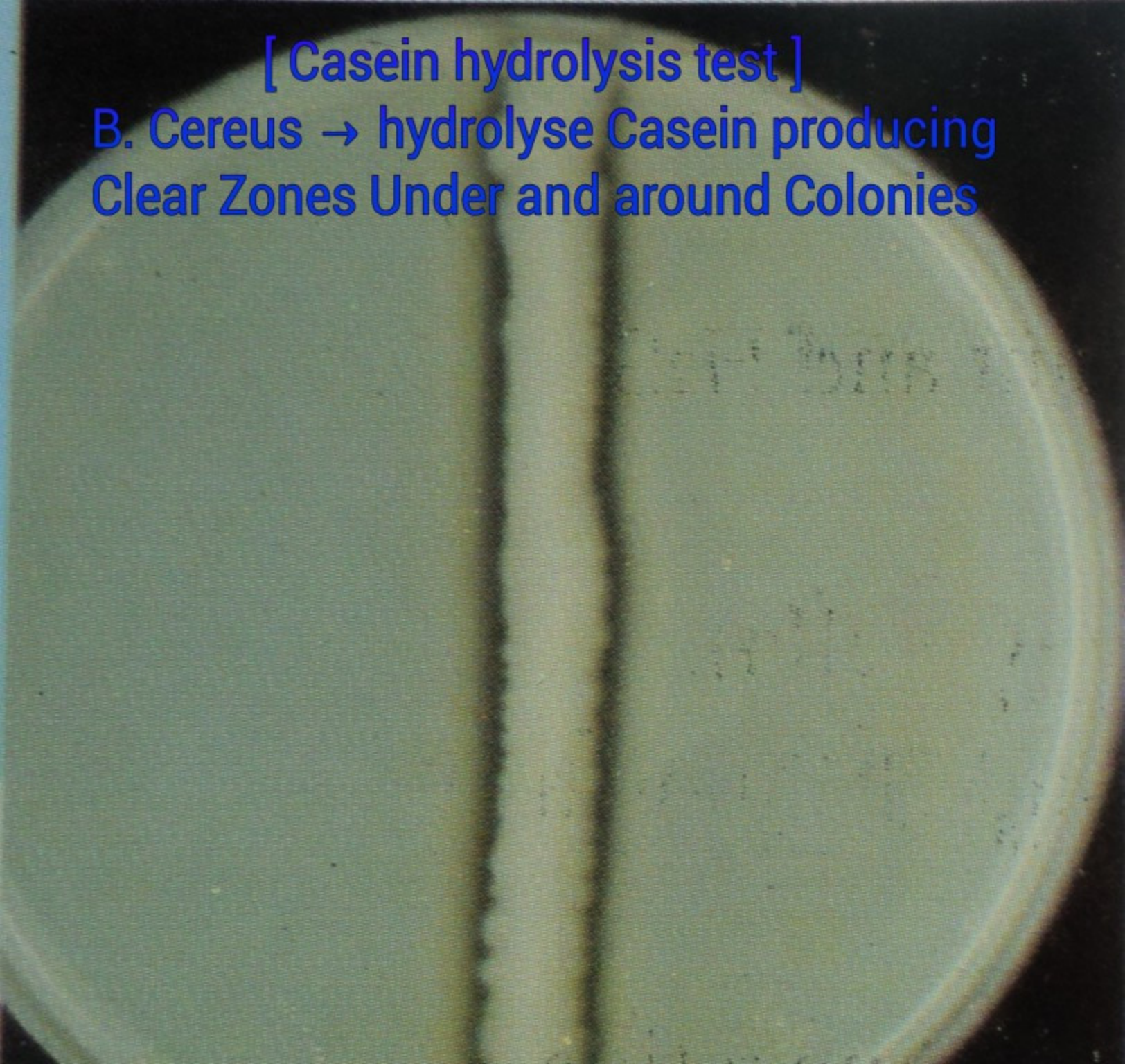
B. sphaericus → not hydrolyse Starch which react with lugol's iodine producing deep blue Colour [left & middle]

B. Cereus → hydrolyse Starch + lugol's iodine giving Brownish yellow Colour [right]



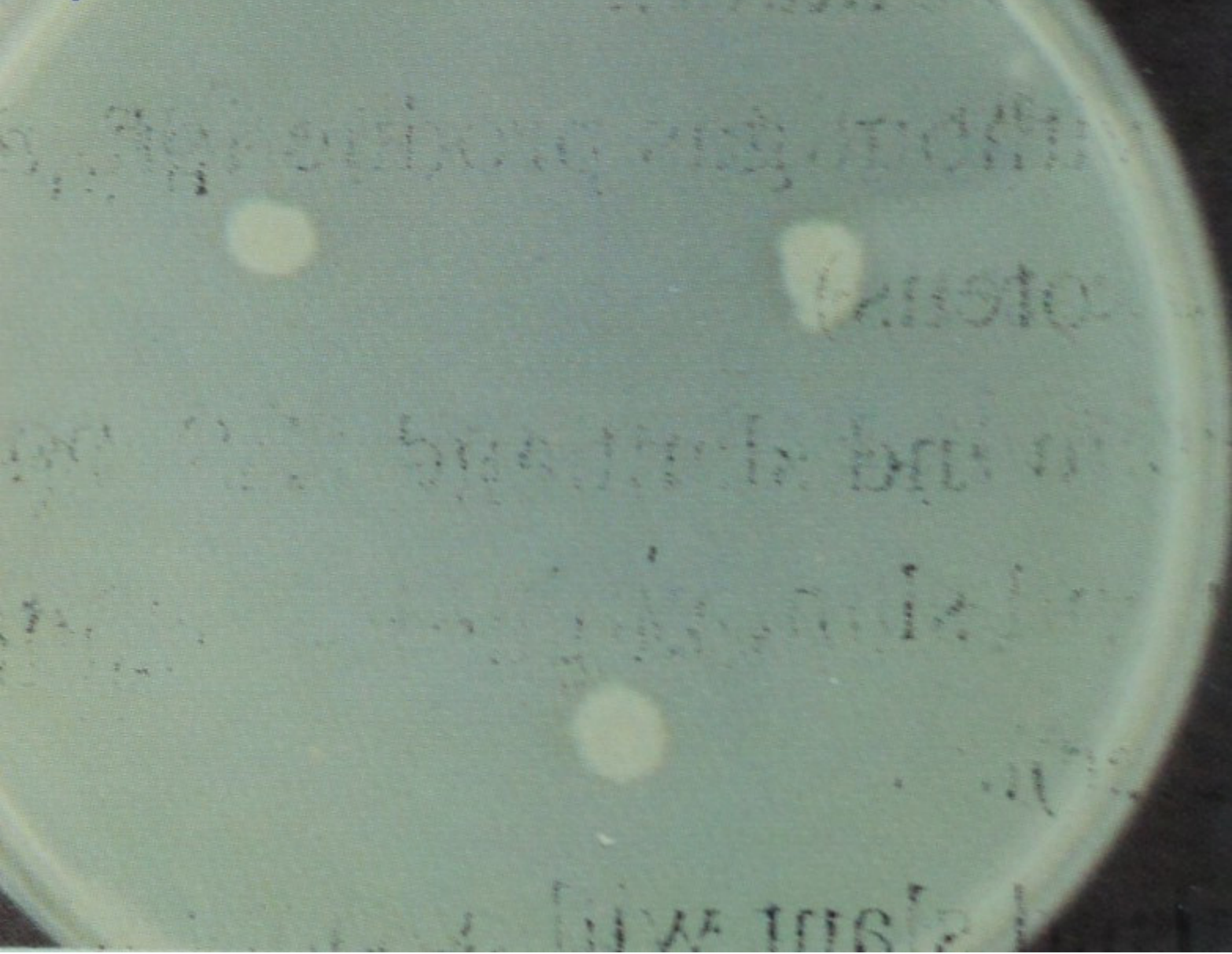
[Casein hydrolysis test]

B. Cereus → hydrolyse Casein producing
Clear Zones Under and around Colonies



[Casein hydrolysis test]

B. Sphaericus → not hydrolyse Casein So, not produce Clear Zones Under or around Colonies



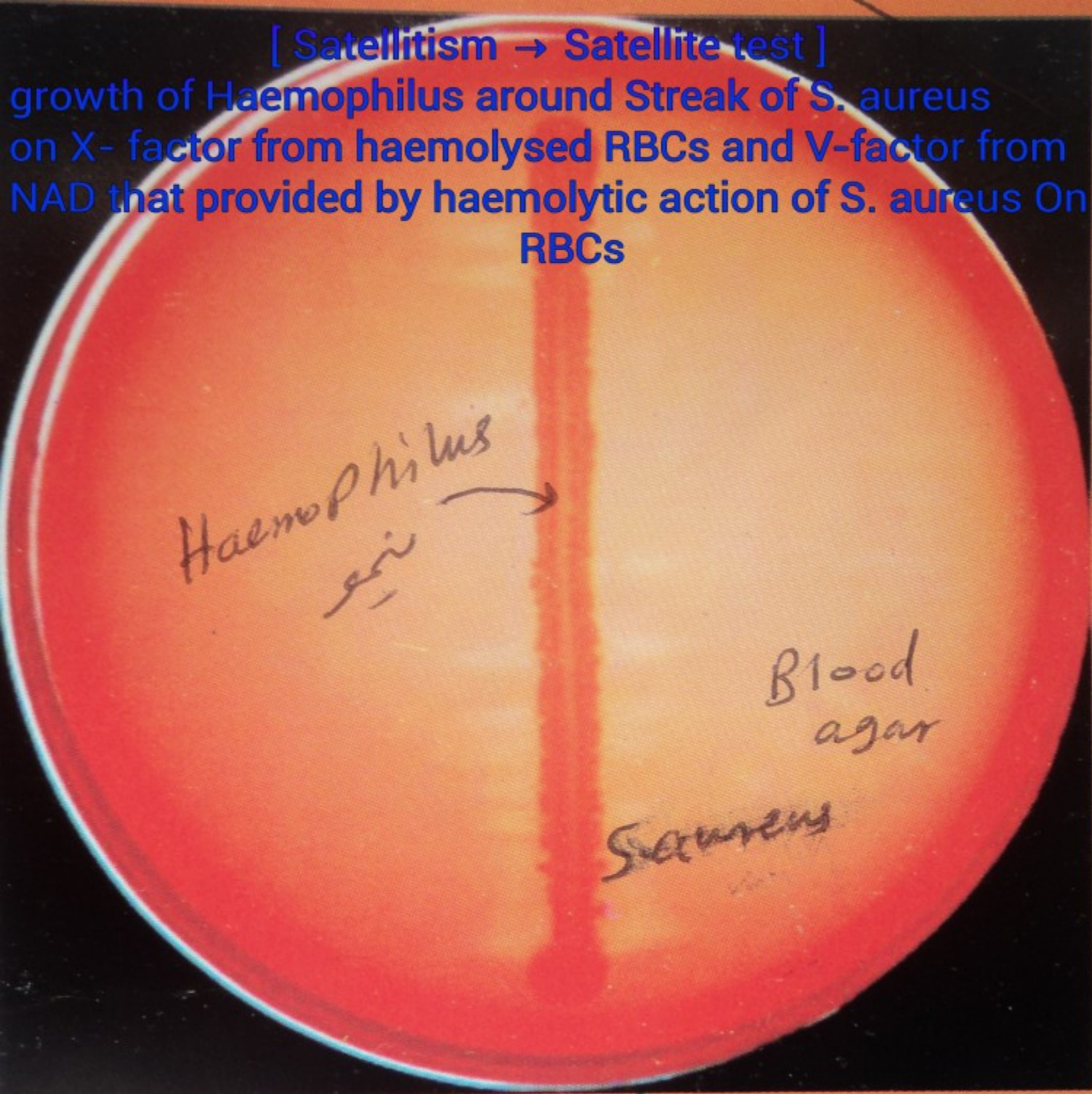


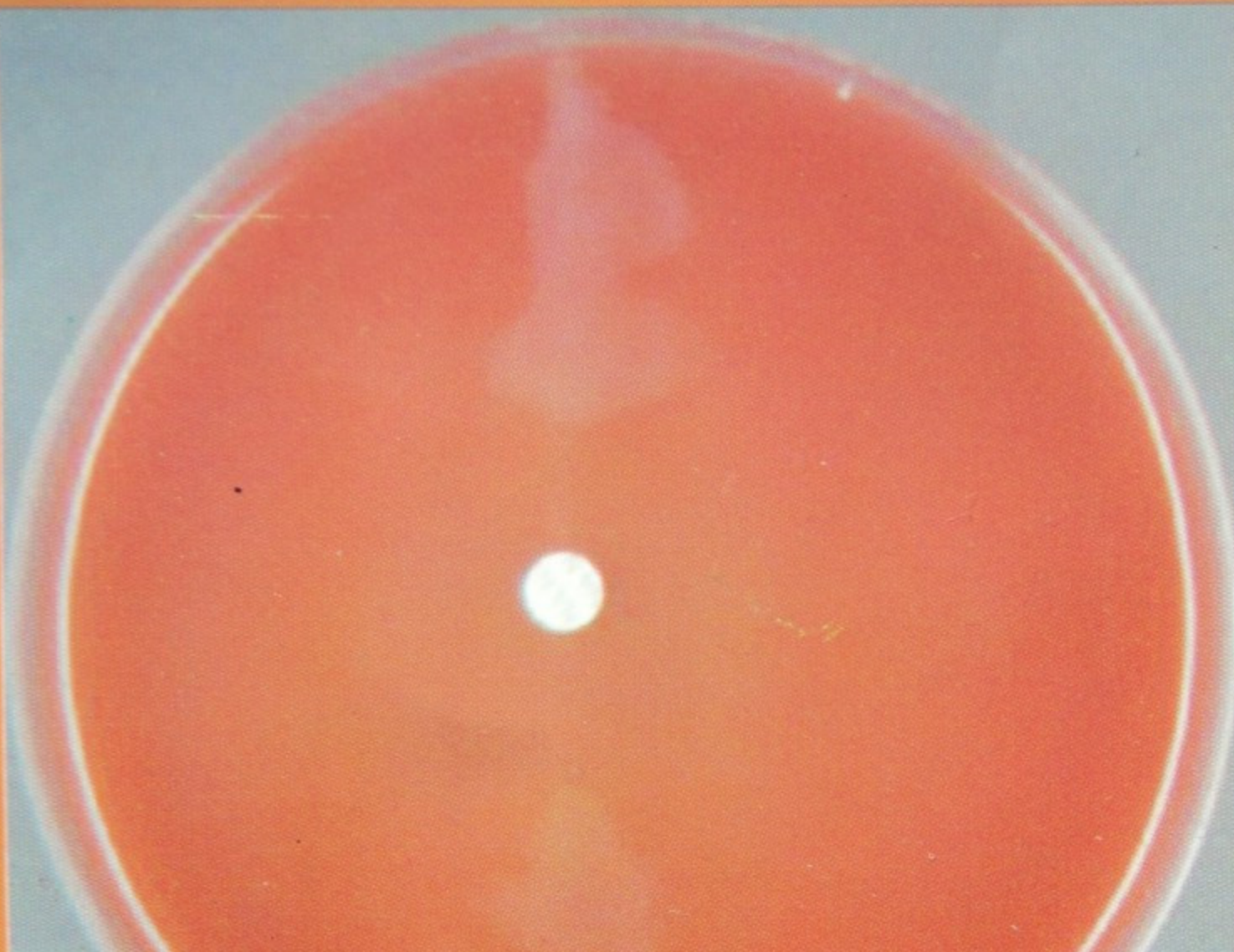
[CAMP test]

S. agalactia produce extracellular Compounds Which Conjugate with Beta haemolysin of S.aureus producing Complete haemolysis of Sheep RBCs on the Blood agar

[Satellitism → Satellite test]

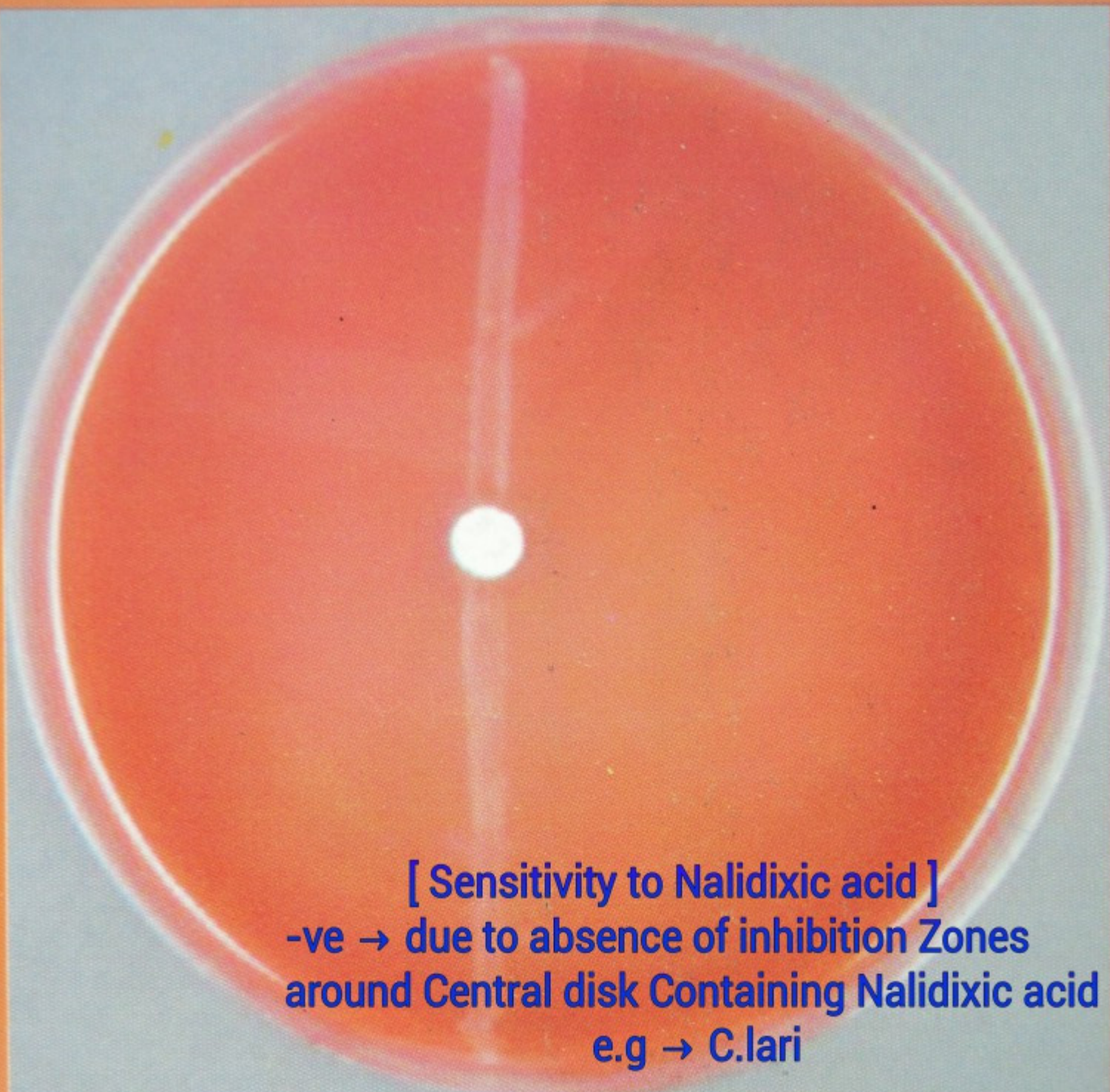
growth of *Haemophilus* around Streak of *S. aureus*
on X- factor from haemolysed RBCs and V-factor from
NAD that provided by haemolytic action of *S. aureus* On
RBCs





[Sensitivity to Nalidixic acid]

**+ve → due to presence of inhibition Zones
around Central disk Containing Nalidixic acid
e.g → C.Jejuni sub.sp jejuni**



[Sensitivity to Nalidixic acid]
-ve → due to absence of inhibition Zones
around Central disk Containing Nalidixic acid
e.g → C.lari

[Sensitivity to O129]

Vibrio Parahaemolyticus Sensitive to O 129



[sensitivity to O129]

Aeromonas hydrophila resist O129





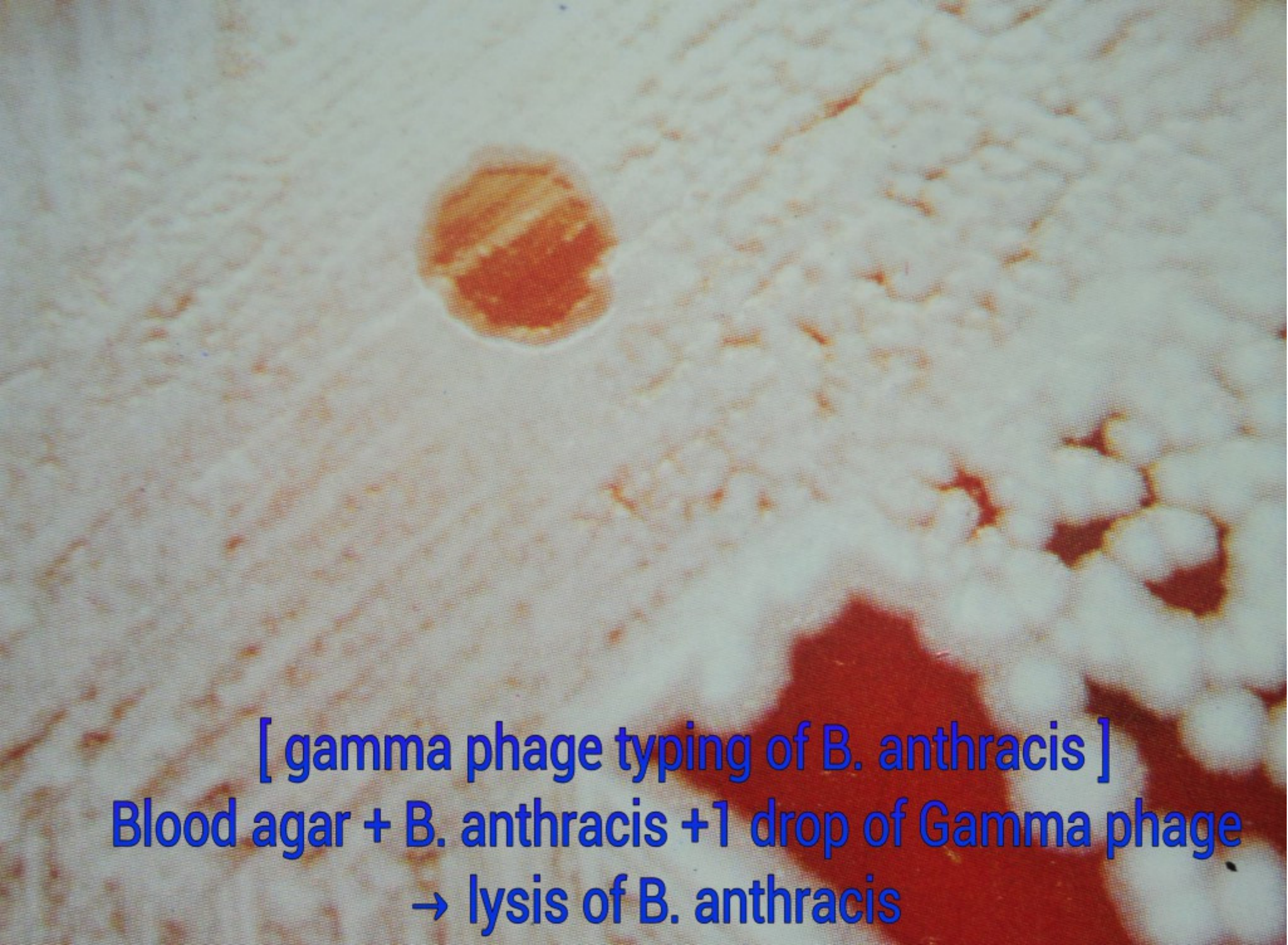
[Optochin test]

S. pneumoniae Sensitive to optochin So , When inoculated on Blood agar with optochin Containing disks not grow around optochin

[Sensitivity to Optochin]

Streptococcus resist optochin SO , grow around
disks Containing Optochin in Blood agar



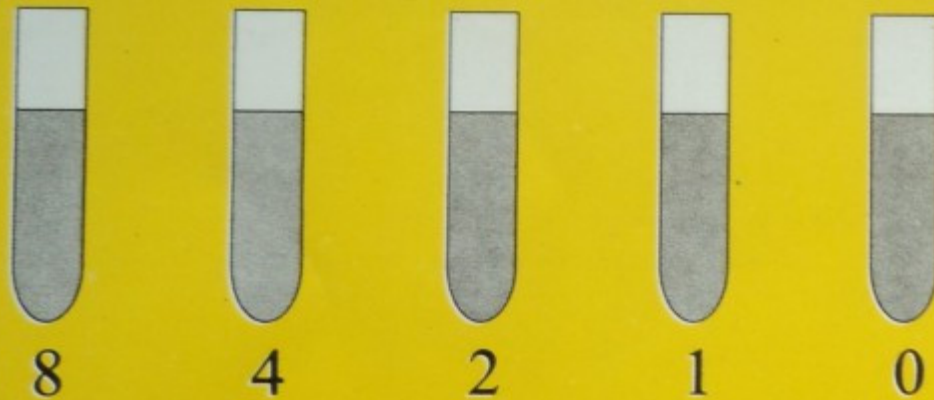


[gamma phage typing of B. anthracis]
Blood agar + B. anthracis +1 drop of Gamma phage
→ lysis of B. anthracis

[Determination of MIC to tetracycline for a tested MO]

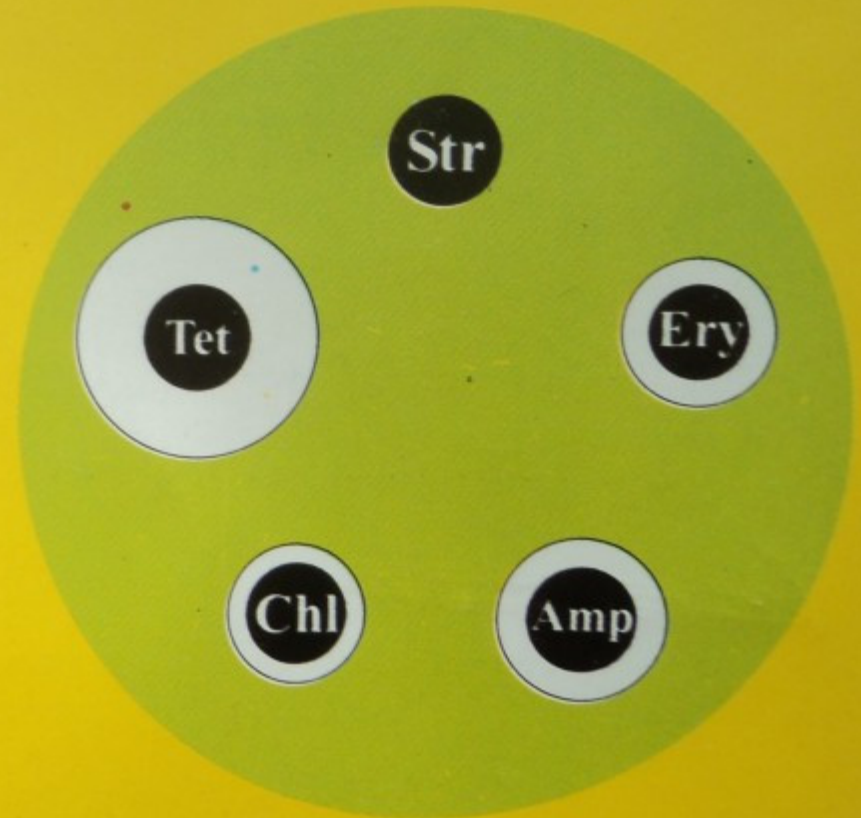
Disk Diffusion Test

Determination of MIC

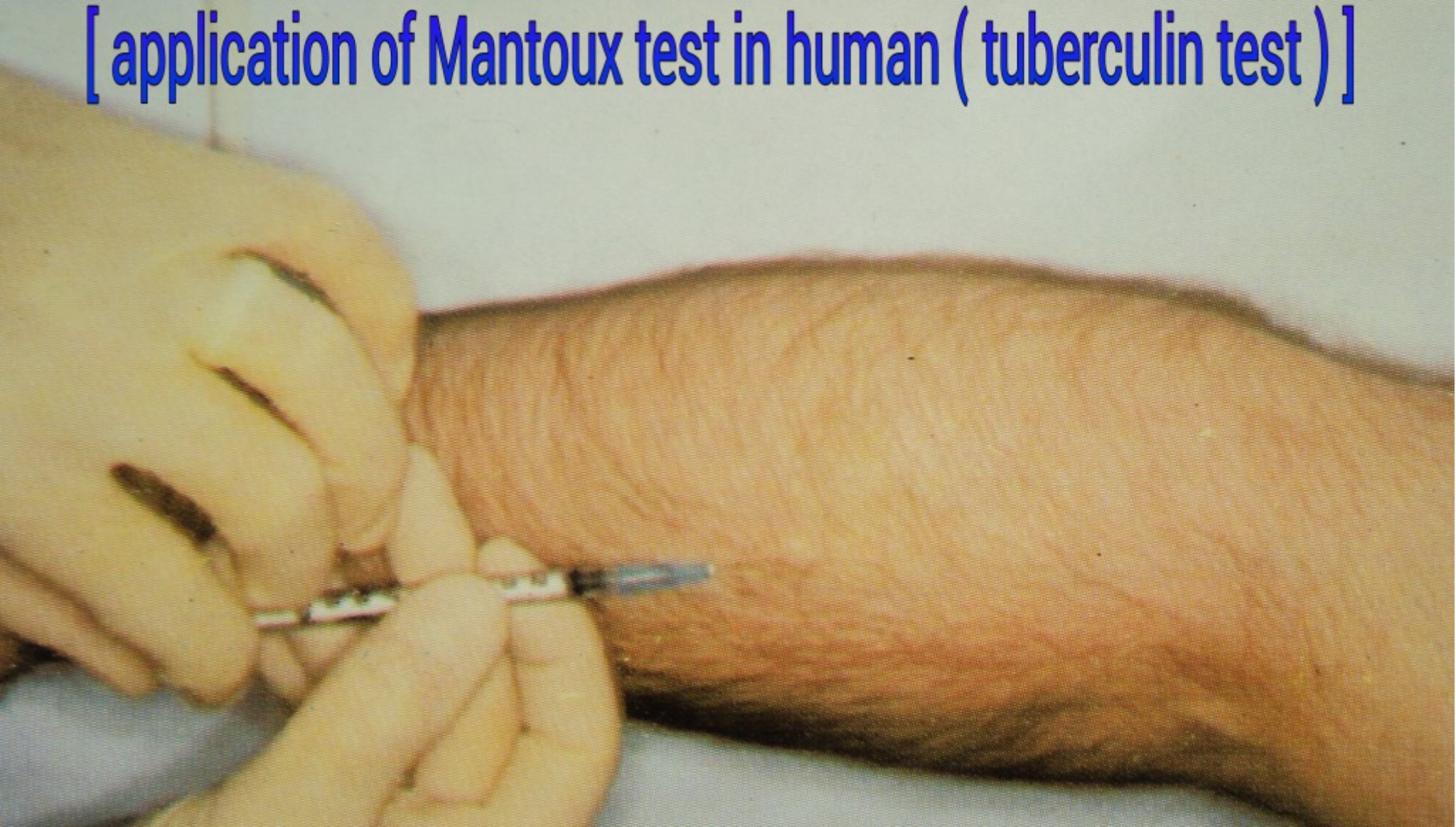


Tetracycline ($\mu\text{g/ml}$)

MIC = 2 $\mu\text{g/ml}$

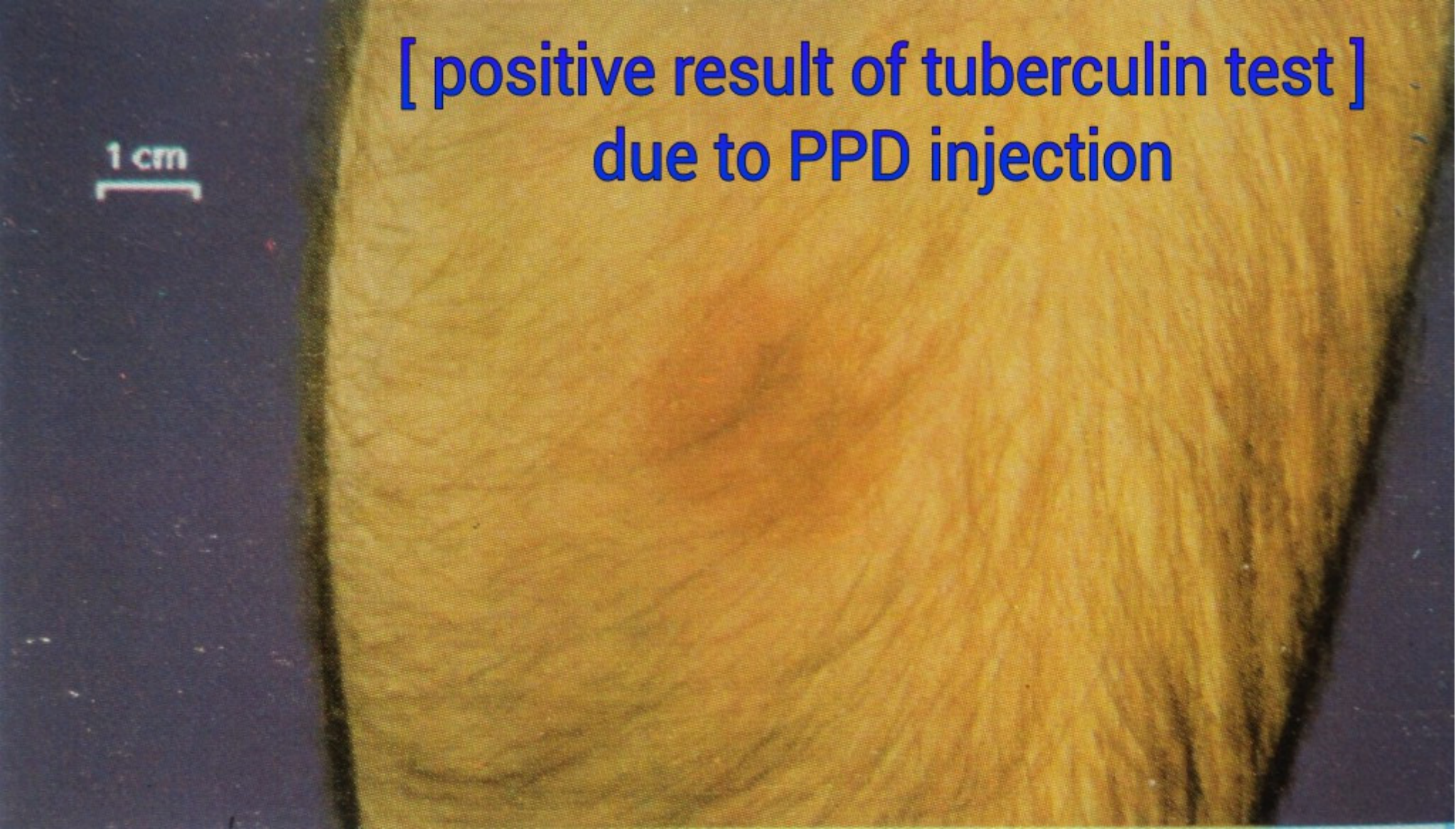


[application of Mantoux test in human (tuberculin test)]



[positive result of tuberculin test]
due to PPD injection

1 cm



[Aesculin hydrolysis test]

MO hydrolyse Aesculin into aesculetin and glucose

Aesculetin + ferric Citrate \rightarrow dark brown phenolic Complex

un inoculated medium

+ve *Enterococcus faecalis*



[CHO fermentation test]

Bromothymol blue as PH indicator

glucose fermented and give acid Or acid and gas

acid detected by indicator by Changing Colour into yellow

gas detected by Derham's tube

Un inoculated
medium

acid

Acid and gas

negative due to
growth of bacteria





[ONPG test]

O-NitroPhenyl Beta D-galactopyranoside hydrolysed
by Beta Galactosidase enzyme into O-Nitrophenol which
is Yellow Colour and Galactose

Un inoculated

positive late
lactose fermenter

Negative



bromothymol blue indicator

[OF test]

oxidation-fermentation test

glucose aerobically → oxidation

anaerobically → fermentation [acid or acid and gas]

indicator → Yellow [oxidation or fermentation]

blue [utilization of Peptone and ammonia
production

un inoculated

Oxidation

fermentation

Gas production

fermentation

Negative
alcaligenes

1

2

3

4

5

6

7

8

9

[Oxidase test]



- Ve

+ ve

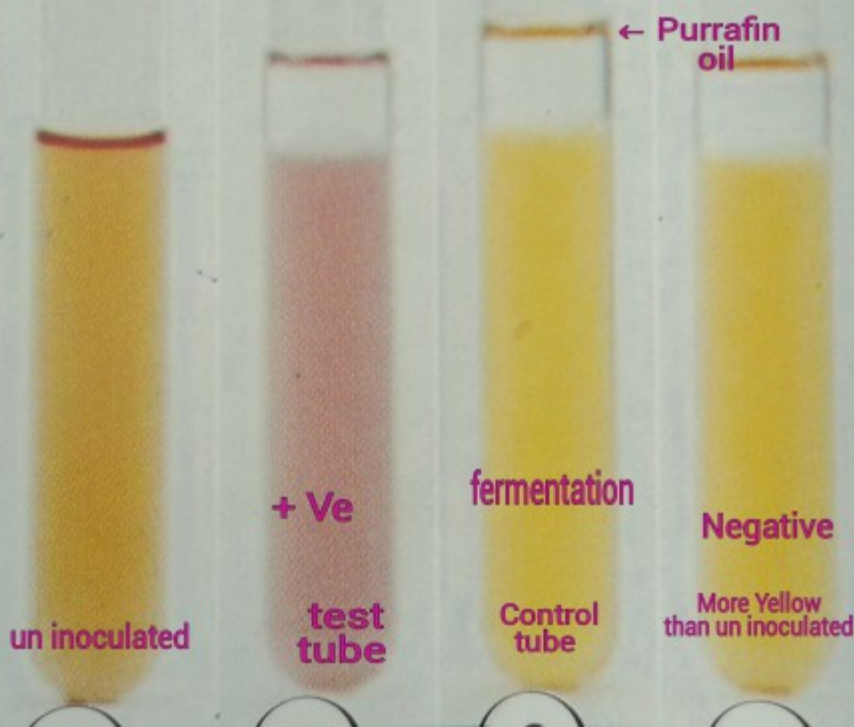
Korack's reagent act as artificial electron acceptor in presence of Cytochrome Oxidase in the suspected Colony in addition to atmospheric $O_2 \rightarrow$ oxidation of the reagent and produce indo Phenol blue [blue]
if not present \rightarrow Colourless

[Decarboxylase test]

Decarboxylation of aa lead to formation of amines make the PH alkaline

PH indicator → Bromocresol purple
violet Colour in alkaline PH
yellow Colour in acid PH

media used → Moller's medium



[Cimmmons Citrate agar]

Citrate utilized producing alKalin PH
detected by Bromothymol blue → [blue]

un
inoculated

+ ve - ve



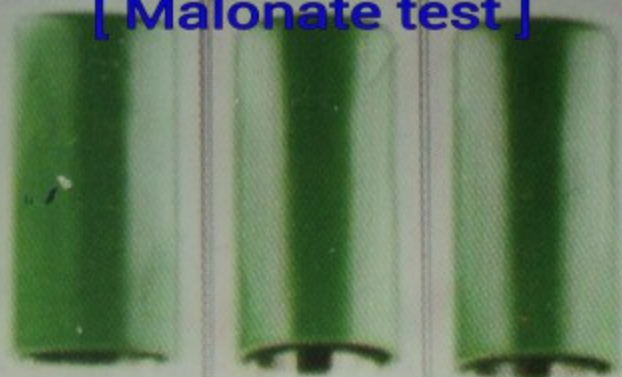
[Hippurate hydrolysis test]
Sodium hippurate hydrolysed by
hippurate hydrolase
into benzoic acid and glycine
glycine + Nihhydrin Solution →
Blue Coloured Complex

- ve

+ ve



[Malonate test]




Malonate is utilised producing
alkaline PH Which detected by
bromothymol blue → [blue]

un
inoculated

+ ve - ve





[Urease test]
urea utilised by Urease enzyme
producing ammonia pink Colour
with phenol red indicator

un
inoculated

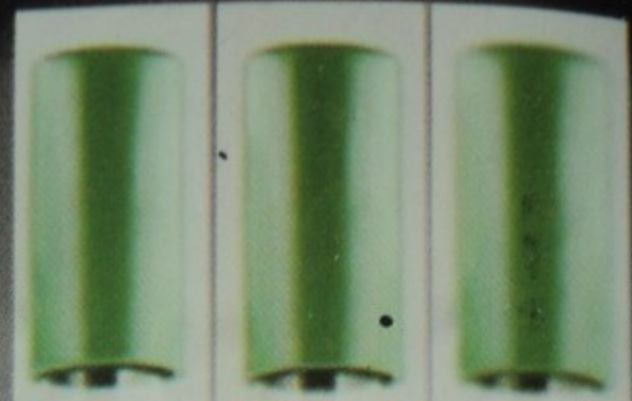
+ve

-ve

1

2

3



[Indole test]

tryptophane hydrolysed by tryptophanase
giving indole which produce red ring with
Kovac's reagent

un
Inoculated

+ Ve

- Ve

1

2

3



[Methyl red test]

glucose utilised giving large Amount of acid
making PH = 4.4 giving red Colour detected
by methyl red indicator

un
inoculated

+ Ve

-Ve

1

2

3



[Voges Proskauer test]

glucose utilised giving acid and acetyls
Methyl Carbinol producing red Colour With
alpha naphthol KOH

un
inoculated

+ ve

- ve

1

2

3

[Phenylalanine Deaminase test]

phenylalanine deaminated giving

phenylpyruvic acid + $\text{FeCl}_3 \rightarrow$ green Colour

un
inoculated

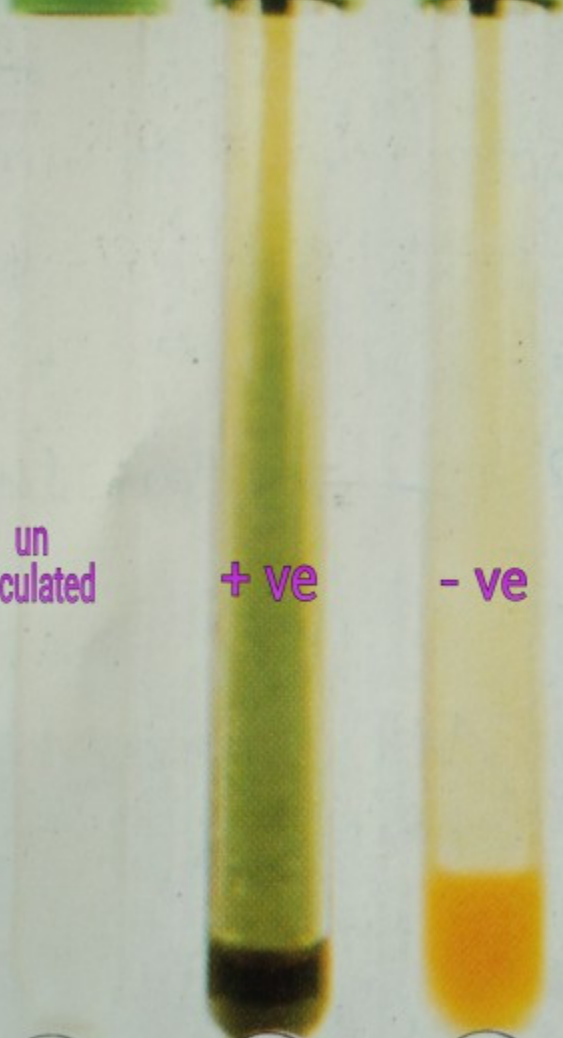
+ ve

- ve

1

2

3



Slant

[lysine iron agar]

glucose & iron & lysine

iron + H_2S → Iron sulphide [Black]

glucose decarboxylated giving Alkaline PH

detected BY Bromocresol Purple and Cresol red

as Violet or purple Colour in Slant

glucose → fermented giving acid [Yellow] in Butt

un
inoculated

Alkaline / H_2S

alkaline / H_2S

1

2

3



[Meta bisulphite reduction]

iron + H₂S → iron Sulphide [Black]

MicroaeroPhylic MO

un
Inoculated

+ ve

- ve



[Proteolysis of Ioeffler's Serum Slopes]

determine Organisms
able to breakdown of
Serum protein

L.M.B



+ ve

- ve

1

2

[tube Coagulase test]
determine if Organism Can
Clot Plasma by Coagulase

0.5 ml Citrated Human Plasma + 0.5 ml Growth
incubated at 37°C for 4-24 hrs

+ve
clot



-ve
No Clot



[Motility test]

Soft agar [Semisolid media]

un
Inoculated

Motile

Non motile

1

2

3

4

[Tripple Sugar iron media] TSI agar

phenol red indicator

iron + H₂S → Iron Sulphide

glucose in butt
lactose, Sucrose in Slant

Gas

No gas

NO
gas

Acid
butt
and
Slant

alkaline
Slant

alkaline
slant

alkaline
butt
and
Slant

acid
Butt
and
Slant

acid
Butt
and
Slant

No
gas

-Ve

Un inoculated

H₂S

H₂S

H₂S

H₂S

gas

No
H₂S

acid
butt

acid
butt

1

2

3

4

5

6

7

8

[Nitrate reduction test] without gas production

Nitrate $\xrightarrow{\text{reduction}}$ Nitrite

↓
Zink dust
orange -Ve
no reduction

Un inoculated

+ Ve

↓
↓
↓
Sulphanilic
acid & a naphthol

Colourless after addition of Sulphanilic acid
and alpha naphthol

Negative

[Nitrate reduction Test] with gas production

reduction of Nitrates
to nitrites and reduction
of nitrites to free N_2
in → right tube

→ Vaseline



N_2

-Ve

+ve

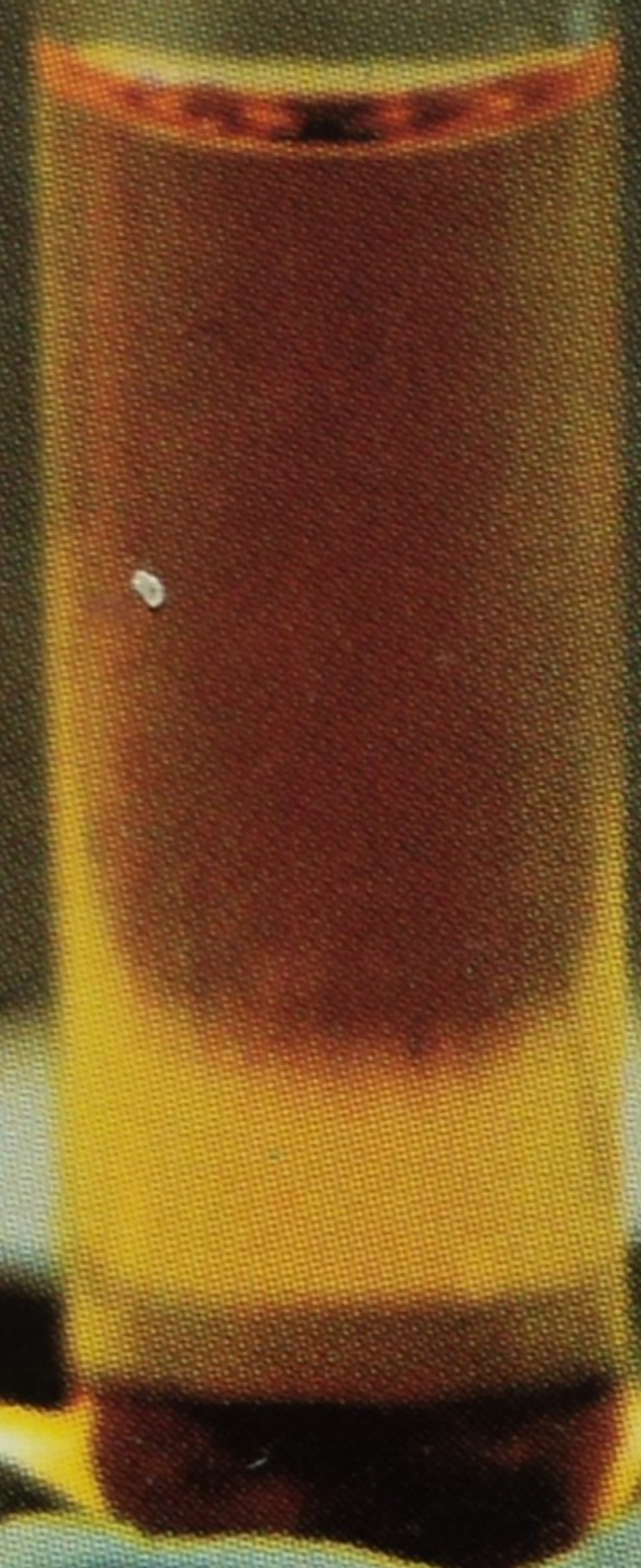
Test

Control

growth on Broth Containing 6.5% NaCl

turbidity ←

✓
xve



Test

Control

→ No turbidity

No growth On Broth Containing 6.5 % Nacl